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(57) Abstract

Protein kinase mutant and wild-type genes encoding polypeptides of the class heretofore designated "casein kinase I" and useful in screening compositions which may effect DNA double-strand break repair activity are disclosed. Also disclosed are methods using the polynucleotides in cell-proliferative disorders.

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PROTEIN KINASES

This application is a Continuation-in-Part of U.S. Application Serial No. 08/008,001, filed January 21, 1993, which is a Continuation-in-Part of U.S. Application Serial No. 728,783, filed July 3, 1991.

FIELD OF THE INVENTION

The present invention relates to novel polynucleotides encoding polypeptides which correspond to the class of protein kinase isolates heretofore referred to as casein kinase I and which possess protein kinase and/or DNA recombination/repair promoting functional capabilities.

BACKGROUND OF THE INVENTION

Protein Kinases

The protein kinases comprise an exceptionally large family of eukaryotic proteins which mediate the responses of cells to external stimuli and are related by amino acid sequence homology within the so-called "catalytic domain" of the enzymes. To date, in excess of 100 unique members of the protein kinase family from a wide variety of eukaryotic organisms have been described and characterized at the amino acid sequence level. See, e.g., Hanks, *et al.* (*Science*, 241:42-52, 1988) which presents a sequence alignment of 65 protein kinase catalytic domains which range in size from about 250 to 300 amino acids and Hanks, *et al.* (*Methods in Enzymol.*, 200:38-62, 1991) presenting a catalytic domain sequence alignment for 117 distinct protein kinase family members including a variety of vertebrate, invertebrate, higher plant and yeast species enzymes. The location of the catalytic domain within a protein kinase is not fixed. In most single subunit enzymes, the domain is near the carboxy terminus of the polypeptide while in multimeric protein kinases the catalytic domain takes up almost the entirety of the subunit polypeptide.

Protein kinases are generally classified into a protein-serine/threonine subfamily or a protein-tyrosine subfamily on the basis of phosphorylation substrate specificity. Among the many classes of enzymes within

the protein-serine/threonine kinase subfamily are two distinct classes which have been designated casein kinase I and casein kinase II based on the order of their elution from DEAE-cellulose. The casein kinases are distinguished from other protein kinases by their ability to phosphorylate serine or threonine residues within acidic recognition sequences such as found in casein. Tuazon, *et al.*, (*Adv. in Second Messenger and Phosphoprotein Res.*, 23:123-164, 1991) presents a review of over 200 publications related to casein kinase I and II, addressing the physicochemical characterization, recognition sequences, substrate specificity and effects on metabolic regulation for these two classes of enzymes. Casein kinase II is active as a heterotetramer and the complete amino acid sequences of human, rat, *Drosophila* and yeast species catalytic regions have been determined. Despite the fact that partially purified casein kinase I preparations have been obtained from cell nuclei, cytoplasm, and cell membranes of various plant and animal species, prior to the present invention, nothing was known concerning the primary structure of its enzymatically active monomeric subunit.

As of the time of the present invention, therefore, there existed a significant need in the art for information concerning the primary structure (amino acid sequence) of protein-serine/threonine kinase enzymes of the casein kinase I class. Such information, provided in the form of DNA sequences encoding one or more of these kinases (from which primary structures could be deduced), would allow for the large scale production of kinases by recombinant techniques as well as for determination of the distribution and function of these enzymes, the structural distinctions between membrane-bound and non-membranous forms, the potential ligand-receptor interactions in which these kinases interact, and the identification of agents capable of modulating ligand-receptor binding, kinase, and other activities.

DNA Recombination And Repair

Chromosomes experience single-stranded or double-stranded breaks as a result of energy-rich radiation, chemical agents, as well as spontaneous breaks occurring during replication among others. Although genes present in the chromosomes undergo continuous damage, repair, exchange, transposition, and

splicing, certain enzymes protect or restore the specific base sequences of the chromosome.

The repair of DNA damage is a complex process that involves the coordination of a large number of gene products. This complexity is in part dependent upon both the form of DNA damage and cell cycle progression. For example, in response to ultraviolet (UV) irradiation, cells can employ photoreactivation or excision repair functions to correct genetic lesions. The repair of strand breaks, such as those created by X-rays, can proceed through recombinational mechanisms. For many forms of DNA damage, the cell is induced to arrest in the G2 phase of the cell cycle. During this G2 arrest, lesions are repaired to ensure chromosomal integrity prior to mitotic segregation.

Since the transfer of genetic information from generation to generation is dependent on the integrity of DNA, it is important to identify those gene products which affect or regulate genetic recombination and repair. Through the use of organisms with specific genetic mutations, the normal functional gene can be obtained, molecularly cloned, and the gene products studied.

In eukaryotes such as *Saccharomyces cerevisiae*, genetic studies have defined repair-deficient mutants which have allowed the identification of more than 30 radiation-sensitive (*RAD*) mutants (Haynes, *et al.*, in *Molecular Biology of the Yeast Saccharomyces*, pp. 371, 1981; J. Game *in Yeast Genetics: Fundamental and Applied Aspects*, pp. 109, 1983). These mutants can be grouped into three classes depending upon their sensitivities. These classes broadly define excision-repair, error-prone repair, and recombinational-repair functions. The molecular characterization of yeast *RAD* genes has increased the understanding of the enzymatic machinery involved in excision repair, as well as the arrest of cell division by DNA damage.

The understanding of RAD genes and their expression products has become increasingly important as research continues to develop more effective therapeutic compositions. Often these new compositions appear quite effective against a particular disease condition, such as certain tumors, but prove to be too toxic for *in vivo* therapy in an animal having the disease. Indeed, these compositions can actually increase the likelihood of mutagenesis.

Most agents that are mutagenic or carcinogenic are in themselves unreactive, but are broken down to reactive intermediates *in vivo*. It is these reactive intermediates which interact with DNA to produce a mutation. This event is thought to be the initial step in chemical carcinogenesis. Mutations in a large number of genes affect the cellular response to agents that damage DNA. In all likelihood, many of these mutated genes encode enzymes that participate in DNA repair systems. Consequently, when the repair system is compromised, the cells become extremely sensitive to toxic agents. Although the DNA may revert to normal when DNA repair mechanisms operate successfully, the failure of such mechanisms can result in a transformed tumor cell which continues to proliferate.

Although there are currently available tests to determine the toxicity or mutagenicity of chemical agents and compositions, there are limitations in both laboratory screening procedures and animal toxicity tests. These limitations include extrapolating laboratory data from animals to humans. There is often a large measure of uncertainty when attempting to correlate the results obtained in laboratory animals with effects in human subjects. In most cases, doses of the test drug have been used in the animal which are too high to be safely administered to humans. In addition, some types of toxicity can be detected if the drug is administered in a particular species, yet may be missed if the experiment is not done in the correct animal species. Moreover, many currently available laboratory tests are incapable of detecting certain types of toxic manifestations which occur in man.

Phenotypic complementation, as a way of identifying homologous normal functional genes, is widely used. For example, the human homologue of the yeast cell cycle control gene, cdc 2, was cloned by expressing a human cDNA library in *Schizosaccharomyces pombe* and selecting those clones which could complement a mutation in the yeast cdc 2 gene (Lee, *et al.*, *Nature*, 327:31, 1987). A mammalian gene capable of reverting the heat shock sensitivity of the $RAS2^{val19}$ gene of yeast, has also been cloned by using complementation (Colicelli, *et al.*, *Proc.Nat'l.Acad.Sci. USA*, 86:3599, 1989). A rat brain cDNA library was used to clone a mammalian cDNA that can complement the loss of

growth control associated with the activated RAS2 gene in yeast. The gene, DPD (dunce-like phosphodiesterase), encodes a high-affinity CAMP phosphodiesterase.

In summary, limitations and uncertainties of existing laboratory tests fail to provide an accurate method of examining the effects of a composition on DNA integrity. In view of this, a considerable need exists for screening methodologies which are inexpensive, rapid, and contain the relevant gene from the animal which is to be treated with the composition. Such methods provide a direct assay to determine if a composition interferes with the DNA repair system of a cell.

SUMMARY OF THE INVENTION

In one of its aspects, the present invention provides purified and isolated polynucleotides (e.g., DNA sequences and RNA transcripts thereof) encoding eukaryotic protein kinases of the casein kinase I class herein designated as "*HRR25*-like" proteins and characterized by greater than 35% amino acid sequence homology with the prototypical yeast enzyme *HRR25* through the protein kinase catalytic domain thereof. Polynucleotides provided by the invention include RNAs, mRNAs and DNAs, including antisense forms thereof. Preferred DNA sequences of the invention include genomic and cDNA sequences as well as wholly or partially chemically synthesized DNA sequences and biological replicas thereof. Specifically illustrating the invention are *Saccharomyces cerevisiae* DNAs including those encoding *HRR25* and NUF1, *Schizosaccharomyces pombe* DNAs including those encoding *Hhp1* + and *Hhp2*+, and human DNAs including those encoding CKIα1Hu, CKIα2Hu, CKIα3Hu, CKIγ1Hu, CKIγ2Hu, and CKIδHu. Also provided are autonomously replicating recombinant constructions such as plasmid and viral DNA vectors incorporating such sequences and especially vectors wherein DNA encoding an *HRR25*-like casein kinase I protein is linked to an endogenous or exogenous expression control DNA sequence.

According to another aspect of the invention, host cells, especially unicellular host cells such as procaryotic and eukaryotic cells, are stably transformed with DNA sequences of the invention in a manner allowing the

desired polypeptides to be expressed therein. Host cells expressing such *HRR25*-like products can serve a variety of useful purposes. To the extent that the expressed products are "displayed" on host cell surfaces, the cells may constitute a valuable immunogen for the development of antibody substances specifically immunoreactive therewith.

Host cells of the invention are conspicuously useful in methods for the large scale production of *HRR25*-like proteins wherein the cells are grown in a suitable culture medium and the desired polypeptide products are isolated from the cells or from the medium in which the cells are grown.

Also comprehended by the present invention are antibody substances (e.g., monoclonal and polyclonal antibodies, single chain antibodies, chimeric antibodies, CDR-grafted antibodies and the like) and other binding proteins which are specific for *HRR25*-like proteins (i.e., non-reactive with protein kinase molecules which are not related by at least 35% homology with *HRR25* through the protein kinase catalytic domain). Antibody substances can be developed using isolated natural or recombinant *HRR25*-like proteins or cells expressing such products on their surfaces. The antibody substances are useful, in turn, for purifying recombinant and naturally occurring *HRR25*-like polypeptides and identifying cells producing such polypeptides on their surfaces. The antibody substances and other binding proteins are also manifestly useful in modulating (i.e., blocking, inhibiting, or stimulating) ligand-receptor binding reactions involving *HRR25*-like proteins. Anti idiotypic antibodies specific for anti-*HRR25*-like antibody substances are also contemplated. Assays for the detection and quantification of *HRR25*-like proteins on cell surfaces and in fluids such as serum and cytoplasmic fractions may involve a single antibody substance or multiple antibody substances in a "sandwich" assay format.

Recombinant *HRR25*-like protein products obtained according to the invention have been observed to display a number of properties which are unique among the eukaryotic protein kinases. As one example, the *HRR25* protein possesses both protein-tyrosine kinase and protein-serine/threonine kinase activities. Moreover, *HRR25* operates to promote repair of DNA strand breaks

at a specific nucleotide sequence and is the only protein kinase known to have such recombination/repair promoting activity.

The DNA sequence information for yeast and mammalian (including human) species *HRR25*-like proteins which is provided by the present invention makes possible the identification and isolation of DNAs encoding other *HRR25*-like proteins by such well-known techniques as DNA/DNA hybridization and polymerase chain reaction (PCR) cloning.

Recombinant *HRR25*-like proteins and host cells expressing the same are useful in screening methods designed to examine the effects of various compositions on DNA break repair and protein kinase activities of the proteins. Protein kinase inhibitory effects may be assessed by well-known screening procedures such as described in Hidaka, *et al.* (*Methods in Enzymology*, 201:328-339, 1991).

BRIEF DESCRIPTION OF THE DRAWING

Further aspects and advantages of the present invention will be apparent upon consideration of the following detailed description of presently preferred embodiments thereof, reference being made to the drawing wherein:

Figure 1 (A) presents an alignment of the predicted amino acid sequence of *HRR25* with the catalytic domains of the yeast CDC28, yeast KSS1 and human RAF1 protein kinases. Figure 1(B) shows a schematic representation of the structure of *HRR25*, and

Figure 2 presents an alignment of the predicted amino acid sequences of *HRR25* with the sequences of three other *Saccharomyces cerevisiae* *HRR25*-like proteins (YCK1/CKI2, YCK2/CKI1, and NUF1), two *HRR25*-like proteins (*Hhp1*+ and *Hhp2*+) from *Schizosaccharomyces pombe* and three putative isoforms (CKIα1Hu, CKIα2Hu, and CKIα3Hu) of a human *HRR25*-like protein.

DETAILED DESCRIPTION OF THE INVENTION

In one of its aspects, the present invention relates to a DNA encoding a recombination/repair promoting polypeptide which can be used in an assay system to examine the effects of various compositions on DNA integrity. These functional sequences, which can be characterized by their ability to promote restoration of DNA strand breaks, permit the screening of compositions to determine whether a particular composition has an effect on the restoration of such repair activity. The invention also provides a DNA sequence encoding a polypeptide which promotes normal mitotic recombination, but is defective in protein kinase activity and essentially unable to repair DNA strand breaks. This defective DNA sequence is highly useful for identifying other DNA sequences which encode proteins with functional protein kinase activity.In addition, the present invention relates to the polypeptide encoded by the defective DNA sequence, as well as the polypeptide encoded by the functional wild-type DNA.

In order to identify a DNA sequence encoding a polypeptide with protein kinase activity, a method is provided whereby a DNA library is screened for nucleotide sequences capable of restoring DNA strand break repair in a mutant lacking such activity. A method is further provided for identifying a composition which affects the activity of a mammalian polypeptide having protein kinase activity, wherein the polypeptide is capable of restoring DNA double-strand break repair activity in a mutant lacking such activity.

In general, the defective protein kinase can be characterized by its ability to promote normal mitotic recombination, while being essentially unable to repair DNA double-strand break including that which occurs at the cleavage site:

CAACAG
GTTGTC

The DNA double-strand breaks which the defective protein kinase is essentially unable to repair can be induced by various means, including endonucleases, x-rays, or radiomimetic agents including alkylating agents. Preferred endonucleases

are those which recognize the same nucleotide cleavage site as endonuclease *HO*. Radiomimetic alkylating agents having methylmethane sulfonate activity are preferred. Those of skill in the art will be able to identify other agents which induce the appropriate DNA strand breaks without undue experimentation.

The present invention specifically discloses mutants sensitive to continuous expression of the DNA double-strand endonuclease *HO*, which codes for a 65 kDa site-specific endonuclease that initiates mating type interconversion (Kostriken, *et al.*, *Cold Spring Harbor Symp.Quant.Biol.*, 49:89, 1984). These mutants are important to understanding the functions involved in recognizing and repairing damaged chromosomes. This invention also discloses a yeast wild-type DNA recombination and repair gene called *HRR25* (*H*O and/or *r*adiation *r*epair). Homozygous mutant strains, *hrr25-1*, are sensitive to methylmethane sulfonate and X-rays, but not UV irradiation. The wild-type gene encodes a novel protein kinase, homologous to other serine/threonine kinases, which appears critical in activation of DNA repair functions by phosphorylation.

The *HRR25* kinase is important for normal cell growth, nuclear segregation, DNA repair and meiosis, and deletion of *HRR25* results in cell cycle defects. These phenotypes, coupled with the sequence similarities between the *HRR25* kinase and the *Raf/c-mos* protein kinase subgroup suggest that *HRR25* might play a similar role in *S. cerevisiae* growth and development. The defects in DNA strand break repair and the aberrant growth properties revealed by mutations in *HRR25* kinase, expands the role that protein kinases may play and places *HRR25* in a functional category of proteins associated with DNA metabolism.

The development of specific DNA sequences encoding protein kinase polypeptides of the invention can be accomplished using a variety of techniques. For example, methods which can be employed include (1) isolation of a double-stranded DNA sequence from the genomic DNA of the eukaryote; (2) chemical synthesis of a DNA sequence to provide the necessary codons for the polypeptide of interest; and (3) *in vitro* synthesis of a double stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell.

In the latter case, a double stranded DNA complement of MRNA is eventually formed which is generally referred to as CDNA.

The novel DNA sequences of the invention include all sequences useful in providing for expression in prokaryotic or eukaryotic host cells of polypeptides which exhibit the functional characteristics of the novel protein kinase of the invention. These DNA sequences comprise: (a) the DNA sequences as set forth in SEQ. I.D. No. 1 or their complementary strands; (b) DNA sequences which encode an amino acid sequence with at least about 35% homology in the protein kinase domain with the amino acid sequences encoded by the DNA sequences defined in (a) or fragments thereof; and (c) DNA sequences defined in (a) and (b) above. Specifically embraced in (b) are genomic DNA sequences which encode allelic variant forms. Part (c) specifically embraces the manufacture of DNA sequences which encode fragments of the protein kinase and analogs of the protein kinase wherein the DNA sequences thereof may incorporate codons which facilitate translation of mRNA. Also included in part (c) are DNA sequences which are degenerate as a result of the genetic code.

The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like.

With the DNA sequences of the invention in hand, it is a routine matter to prepare, subclone, and express smaller DNA fragments from this or a corresponding DNA sequences. The term "polypeptide" denotes any sequence of amino acids having the characteristic activity of the mutant or wild-type protein kinase of the invention, wherein the sequence of amino acids is encoded by all or part of the DNA sequences of the invention.

The polypeptide resulting from expression of the DNA sequence of the invention can be further characterized as being free from association with other eukaryotic polypeptides or other contaminants which might otherwise be associated with the protein kinase in its natural cellular environment.

Isolation and purification of microbially expressed polypeptides provided by the invention may be by conventional means including, preparative chromatographic separations and immunological separations involving monoclonal and/or polyclonal antibody preparation.

In general, recombinant expression vectors useful in the present invention contain a promotor sequence which facilitates the efficient transcription of the inserted eukaryotic genetic sequence. The expression vector typically contains an origin of replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed cells. The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The polypeptides of the invention can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions.

The DNA sequences of the present invention can be expressed *in vivo* in either prokaryotes or eukaryotes. Methods of expressing DNA sequences containing eukaryotic coding sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors used to incorporate DNA sequences of the invention, for expression and replication in the host cell are well known in the art. For example, DNA can be inserted in yeast using appropriate vectors and introducing the product into the host cells. Various shuttle vectors for the expression of foreign genes in yeast have been reported (Heinemann, *et al., Nature*, 340:205, 1989; Rose, *et al., Gene*, 60:237, 1987). Those of skill in the art will know of appropriate techniques for obtaining gene expression in both prokaryotes and eukaryotes, or can readily ascertain such techniques, without undue experimentation.

Hosts include microbial, yeast, insect and mammalian host organisms. Thus, the term "host" is meant to include not only prokaryotes, but also such eukaryotes such as yeast, filamentous fungi, as well as plant and animal cells which can replicate and express an intron-free DNA sequence of the invention. The term also includes any progeny of the subject cell. It is understood that not all progeny are identical to the parental cell since there may

be mutations that occur at replication. However, such progeny are included when the terms above are used.

Transformation with recombinant DNA may be carried out by conventional techniques well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method using procedures well known in the art. Alternatively, $MgCl_2$ or RbCl could be used in the reaction. Transformation can also be performed after forming a protoplast of the host cell.

Where the host is a eukaryote, various methods of DNA transfer can be used. These include transfection of DNA by calcium phosphate-precipitates, conventional mechanical procedures such as microinjection, insertion of a plasmid encased in liposomes, spheroplast electroporation, salt mediated transformation of unicellular organisms or the use of virus vectors.

Analysis of eukaryotic DNA has been greatly simplified since eukaryotic DNA can be cloned in prokaryotes using vectors well known in the art. Such cloned sequences can be obtained easily in large amounts and can be altered *in vivo* by bacterial genetic techniques and *in vitro* by specific enzyme modifications. To determine the effects of these experimentally induced changes on the function and expression of eukaryotic genes, the rearranged sequences must be taken out of the bacteria in which they were cloned and reintroduced into a eukaryotic organism. Since there are still many functions in eukaryotic cells which are absent in prokaryotes, (e.g., localization of ATP-generating systems to mitochondria, association of DNA with histones, mitosis and meiosis, and differentiation of cells), the genetic control of such functions must be assessed in a eukaryotic environment. Cloning genes from other eukaryotes in yeast has been useful for analyzing the cloned eukaryotic genes as well as other yeast genes. A number of different yeast vectors have been constructed for this purpose. All vectors replicate in *E. coli*, which is important for amplification of the vector DNA. All vectors contain markers, e.g., LEU 2, HIS 3, URA 3, that can be selected easily in yeast. In addition, these vectors also carry antibiotic resistance markers for use in *E. coli*.

Many strategies for cloning human homologues of known yeast genes are known in the art. These include, but are not limited to: 1) low stringency hybridization to detect shared nucleotide sequences; 2) antibody screening of expression libraries to detect shared structural features; and 3) complementation of mutants to detect genes with similar functions.

For purposes of the present invention, protein kinases which are homologous can be identified by structural as well as functional similarity. Structural similarity can be determined, for example, by assessing amino acid homology or by screening with antibody, especially a monoclonal antibody, which recognizes a unique epitope present on the protein kinases of the invention. When amino acid homology is used as criteria to establish structural similarity, those amino acid sequences which have homology of at least about 35% in the protein kinase domain with the prototypical *HRR25* protein are considered to uniquely characterize polypeptides.

Conserved regions of amino acid residues in *HRR25* can be used to identify *HRR25*-like genes from other species. Conserved regions which can be used as probes for identification and isolation of *HRR25*-like genes (homologues) include the nucleotides encoding amino acid sequences GPSLED (amino acids 86 to 91 in SEQ ID NO: 2), RDIKPDNFL (amino acids 127 to 135 in SEQ ID NO: 2), HIPYRE (amino acids 164 to 169 in SEQ ID NO: 2), and SVN (amino acids 181 to 183 in SEQ ID NO: 2), for example. These conserved motifs can be used, for example, to develop nucleotide primers to detect other *HRR25*-like genes by methods well known to those skilled in the art, such as polymerase chain reaction (PCR).

When homologous amino acid sequences are evaluated based on functional characteristics, then a homologous amino acid sequence is considered equivalent to an amino acid sequence of the invention when the homologous sequence is essentially unable to repair (in the case of the repair defective mutant gene) or able to repair (in the case of the natural gene), DNA double-strand breaks, including that which occurs at a nucleotide cleavage site

CAACAG
GTTGTC

and when the homologous amino acid sequence allows normal mitotic recombination.

This invention provides screening methods whereby genes are cloned from plasmid libraries by complementation of a recessive marker. A recipient strain such as *Saccharomyces cerevisiae* is constructed that carries a recessive mutation in the gene of interest. This strain is then transformed with a plasmid, for example, pYES2 (Invitrogen, San Diego, CA) containing the wild-type genomic DNA or cDNA. The clone carrying the gene of interest can then be selected by replica plating to a medium that distinguishes mutant from wild-type phenotypes for the gene of interest. The plasmid can then be extracted from the clone and the DNA studied. Several yeast vectors allow the application of complementation systems to go beyond isolation of yeast genes. Genes from a wide variety of species can be isolated using these vectors. In such systems, DNA sequences from any source are cloned into a vector and can be screened directly in yeast for activities that will complement specific yeast mutations.

In a preferred embodiment, the present invention uses a mutation in yeast, the *hrr25* mutation, which was identified by sensitivity to DNA double-strand breaks induced by the HO endonuclease. The genomic DNA which complements this mutation was isolated by transforming the *hrr25* strain with a DNA library and subsequently screening for methylmethane sulfonate (MMS) resistance. Alternately, functional genes from a variety of mammalian species can now be cloned using the system described.

Yeast genes can be cloned by a variety of techniques, including use of purified RNA as hybridization probes, differential hybridization of regulated RNA transcripts, antibody screening, transposon mutagenesis, cross suppression of mutant phenotypes, cross hybridization with heterologous CDNA or oligonucleotide probes, as well as by complementation in *E. coli*.

Minor modifications of the primary amino acid sequence may result in proteins which have substantially equivalent or enhanced activity as compared to the sequence set forth in SEQ. I.D. NO. 2. The modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous by *HRR25* producing organisms. All of these modifications are included in the invention as long as *HRR25* activity is retained. Substitution of an aspartic acid residue for a glycine acid residue at position 151 in the sequence shown in SEQ. I.D. NO. 2 identifies the mutant *hrr25.*

Antibodies provided by the present invention are immunoreactive with the mutant polypeptides and/or the naturally occurring protein kinase. Antibody which consist essentially of numerous monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibody is made from antigen containing fragments of the polypeptide by methods well known in the art (Kohler,G. *et al.*, *Nature* 256:495, 1975; *Current Protocols in Molecular Biology*, Ausubel, F. *et al.*, ed.,1989).

The invention also discloses a method for identifying a composition which affects the activity of a polypeptide having tyrosine kinase activity. The polypeptide is capable of promoting restoration of DNA double-strand break repair activity in host cells containing the *hrr25* gene. The composition and the polypeptide are incubated in combination with host cells for a period of time and under conditions sufficient to allow the components to interact, then subsequently monitoring the change in protein kinase activity, for example, by decreased repair of DNA double-strand breaks. The DNA strand breaks are induced, for example, by a radiomimetic agent, such as methylmethane sulfonate, x-rays, or by endonuclease like *HO*. Other means of inducing double-strand breaks that are well known in the art may be employed as well.

One embodiment of the invention provides a method of treating a cell proliferative disorder associated with or *HRR25* or an *HRR25*-like protein comprising administering to a subject with the disorder, a therapeutically effective amount of reagent which modulates an *HRR25*-like protein activity. The term "cell proliferative disorder" denotes malignant as well as non-malignant cell

populations which differ from the surrounding tissue both morphologically and/or genotypically. Such disorders may be associated, for example, with abnormal expression of *HRR25*-like protein genes. "Abnormal expression" encompasses both increased or decreased levels of expression as well as expression of mutant forms such that the normal function of *HRR25*-like genes is altered. Abnormal expression also includes inappropriate temporal expression during the cell cycle or expression in an incorrect cell type. Antisense polynucleotides of the invention are useful in treating malignancies of the various organ systems. Essentially, any disorder which is etiologically linked to altered expression of *HRR25*-like genes is a candidate for treatment with a reagent of the invention. "Treatment" of cell proliferative disorder refers to increasing or decreasing populations of malignant or non-malignant cells.

As used herein, the term "modulate" envisions the suppression of *HRR25*-like protein expression or the augmentation of expression. When a cell proliferative disorder is associated with *HRR25*-like gene overexpression, appropriate reagents such as antisense or binding antibody can be introduced to a cell. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific *HRR25*-like protein mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme. Alternatively, when a cell proliferative disorder is associated with insufficient *HRR25*-like protein, a sense polynucleotide sequence (the DNA coding strand) or *HRR25*-like polypeptide can be introduced into the cell by methods known in the art.

As used herein, the term "therapeutically effective" refers to that amount of polynucleotide, antibody or polypeptide that is sufficient to ameliorate the *HRR25*-associated disorder. "Ameliorate" denotes a lessening of the detrimental effect of the *HRR25*-associated disorder in the subject receiving therapy.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. This

interferes with the translation of the mRNA since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause non-specific interference with translation than larger molecules when introduced into the target *HRR25* producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J. Amer. Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because ribosomes are sequence-specific, only mRNAS with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and longer recognition sequences are preferable to shorter recognition sequences.

The present invention also provides gene therapy for the treatment of cell proliferative disorders which are mediated by *HRR25*-like polypeptides. Such therapy comprises introducing into cells of subjects having the proliferative disorder, the *HRR25*-like antisense polynucleotide. Delivery of antisense polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Disorders associated with under-expression of *HRR25* can similarly be treated using gene therapy with nucleotide coding sequences.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA

virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting an *HRR25*-like sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the *HRR25*-like antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include but are not limited to Ψ2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag, pol* and *env*, by conventional calcium phosphate transfection. These cells are then

transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for *HRR25*-like antisense polynucleotides comprises a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al., Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, *et al., Biotechniques*, 6:682, 1988).

The targeting of liposomes has been classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of

the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

In general, the compounds bound to the surface of the targeted delivery system will be ligands and receptors which will allow the targeted delivery system to find and "home in" on the desired cells. A ligand may be any compound of interest which will bind to another compound, such as a receptor.

The present invention will be better understood upon consideration of the following illustrative examples wherein: Example 1 addresses isolation of *hrr25* mutant strains of *Saccharomyces cerevisiae*; Example 2 describes the isolation of HRR25 DNA by complementation screening; Example 3 is drawn to characterization of the DNA and putative amino acid sequence of HRR25; Example 4 addresses microscopic analysis of HRR25 wild type and hrr25 mutant yeast morphology; Example 5 addresses the relationship of the amino acid sequence of HRR25 and three exemplary protein kinases which are not HRR25-like; Example 6 describes the isolation of DNAs encoding two *Schizosaccharomyces pombe* HRR25-like protein kinases; Example 7 is directed to isolation of DNA encoding another *Saccharomyces cerevisiae* protein, NUF1; Example 8 is drawn to isolation of DNAs encoding various eukaryotic species HRR25-like proteins including three human isoforms, CKIα1Hu, CKIα2Hu, and CKIα3Hu; Examples 9 and 10 are respectively directed to determination of casein kinase and both serine-threonine kinase and tyrosine kinase activities for HRR25; Example 11 is drawn to the recombinant expression of *HRR25* products and the generation of antibodies thereto; Example 12 relates to the isolation of human CKI isoforms, CKIγ1Hu and CKIγ2Hu; Example 13 addresses isolation of another human isoform CKIδHu; Example 14 describes complementation of yeast CKI mutants with human CKI isoforms; and Example 15 is directed to generation of monoclonal antibodies against peptide fragments of human CKIαHu isoforms.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

Example 1

Isolation of *hrr25*

S. cerevisiae strain K264-5B (*MATα ho ura3 can1*^{R} *tyr1 his7 lys2 ade5 met13 trp5 leu1 ade5*) was employed for the mutant isolation. The yeast were transformed according to standard procedures with a *URA3*-based integrating plasmid that contained a *GAL1,10*-regulated *HO* endonuclease and a transformant was mutagenized to approximately 50% survival with ethyl methanesulfonate (EMS), as described (*Current Protocols in Molecular Biology, supra*). The culture was spread onto glycerol-containing rich medium (YPG, to avoid petites), colonies were allowed to form at 30°C, and plates were replicated to glucose (*HO* repressing) and galactose (*HO* inducing) media. Mutants were identified by their inability to grow on galactose. Approximately 200 mutants were chosen for initial characterization and 62 maintained the *gal-* phenotype through repeated single colony purification. Among these, many were not complemented by various *gal* mutants. The remainder (25 mutants) were surveyed for overlapping DNA repair defects by determining sensitivity to ultraviolet (UV) irradiation and to methyl methane sulfonate (MMS). This screening method identified five alleles of known *rad* mutations and one new mutation. This new mutation *hrr25-1* (*H*O and/or *r*adiation *r*epair), presented severe defects and was studied further.

A recessive DNA repair defect is conferred by *hrr25-1* that includes sensitivity to MMS. *Hrr25-1* strains also show sensitivity at 5-20 Krad X-irradiation similar to that observed with mutations in the radiation repair genes *RAD50* and *RAD52* (Cole, *et al., Mol.Cell.Biol.*, 9:3101, 1989). The *hrr25-1* strains are no more sensitive to UV irradiation than wild type and are not temperature sensitive for growth at 37°C. Unlike hypo- and hyper-rec *rad* mutants which have several of the *hrr25-1* phenotypes, *hrr25-1* strains undergo normal mitotic recombination (Cole, *et al., Mol.Cell.Biol.*, 9:3101, 1989). Spontaneous gene conversion and crossing-over were the same for homozygous

hrr25-1 and wild type strains. However, *HRR25* is required for the correct completion of meiosis. The *hrr25-1* homozygotes showed less than 1% spores (tetranucleate cells) under conditions that produced 75-80% spores in an isogenic wild type strain. The *hrr25-1* mutation could be complemented by a number of radiation sensitive mutations (*rad6, 50, 52, 54, and 57*) that present some of the *hrr25* phenotypes, suggesting that *hrr25-1* is a newly uncovered *rad*-like mutation and not one of these previously described genes. These results also indicate that *HRR25* plays a role in DNA repair and meiosis, but is not specifically required for the repair of spontaneous mitotic lesions by recombination.

Example 2

Isolation of *HRR25*

The *HRR25* gene was obtained by complementing for MMS sensitivity using a yeast genomic library constructed in the plasmid YCp50 (Rose, *et al.*, *Gene*, 60:237, 1987). An *hrr25-1* strain, MHML 3-36d (ura3 *hrr25*), was transformed by standard methods (Nickoloff, *et al.*, *J.Mol.Biol.*, 207:527, 1989) to uracil prototrophy, transformants were amplified on media without uracil and replicated to media containing 0.01% MMS. Among 1200 transformants, a single MMS resistant isolate was identified. Complementation for MMS sensitivity was found to segregate with the plasmid as determined by methods known in the art.

A 12 kb genomic fragment was identified and complementing activity was localized to a 3.1 kb *Bam*HI-*Sal*I fragment by transposon mutagenesis and subcloning. This region complemented DNA repair defects as well as meiotic deficiencies. Gene targeting experiments linked this cloned region to *hrr25-1*. Transposon insertion mutations within the *Bam*HI-*Sal*I fragment replaced into the cognate *HRR25* genomic locus did not complement *hrr25-1* for MMS sensitivity, whereas adjacent chromosomal insertions outside the complementing region segregated in repulsion when crossed against *hrr25-1*.

Mini-Tn*10LUK* transposons (Huisman, *et al.*, *Genetics*, 116:191, 1987) were used to delineate the approximate location of *HRR25* on the 12 kb *Bam*HI-*Sal*I fragment. Insertions located to the left hand 9 kb (of the 12 kb genomic fragment) did not inactivate complementation of *hrr25-1* MMS resistance

compared with the un-mutagenized plasmid. Two insertions, located near an *Eco*RV site in the right hand 2 kb inactivated complementation. *HRR25* complementation activity was localized to a 3.4 kb *Sal*I fragment. Approximately 300 bp of this fragment (right hand side of the 12 kb) were part of the pBR322 tetracycline resistance gene (between the *Bam*HI site of PBR322-based YCp50). The *HRR25* open reading frame spans an internal region across an *Eco*RV site and two *Bgl*II sites within the right terminal 3 kb.

The DNA sequence of the 3.1 kb fragment revealed a centrally located open reading frame of 1482 nucleotide. A transposon insertion mutation in this open reading frame inactivated *HRR25* complementation whereas insertions elsewhere in the 12 kb clone did not affect *HRR25* complementation. Transposon-mediated disruption of *HRR25* also revealed several phenotypes not seen with *hrr25-1*. As expected, a Tn*10*-based LUK transposon insertion (Huisman, *et al.*, *Genetics*, 116:191, 1987) into the middle of plasmid-borne *HRR25* coding region inactivated complementation for MMS sensitivity. Transplacement of this insertion into the genomic *HRR25* gene revealed a severe growth defect in addition to MMS sensitivity and meiotic inviability. This severe growth defect was not observed with *hrr25-1* strains. Wild type *HRR25* strains doubled in rich media at 30°C every 80-90 minutes whereas isogenic *hrr25::LUK* strains and *hrr25*Δ doubled every 9-12 hours. *hrr25-1* had a doubling time of 2-4 hours.

To determine whether the mutant phenotypes revealed by the *hrr::LUK* disruption allele represent a null phenotype, the entire *HRR25* coding sequence was deleted. Briefly, deletion of the *HRR25* coding sequence employed a *hisG::URA3::hisG* cassette (Alani, *et al.*, *Genetics*, 116:541, 1988). The 3.1 kb *HRR25 Sal*I fragment was cloned into pBluescript (Stratagene, La Jolla, CA). This plasmid was digested with *Bgl*II and the two *Bgl*II fragments that span the entire *HRR25* gene and its flanking sequences were deleted. Into this deletion was introduced the 3.8 kb BamHI-*Bgl*II *hisG::URA3::hisG* fragment from pNKY51 to create the *hrr25*Δ allele. *Sal*I digestion yielded a linearized fragment that deleted the entire *HRR25* locus. Yeast carrying the deletion-disruption allele (*hrr25*Δ) showed phenotypes identical to those with the *hrr25::LUK* allele for all properties examined, including MMS sensitivity, slow growth, and the sporulation defect,

indicating that wild-type *HRR25* protein is associated with these processes and that the *hrr25::LUK* allele does not indirectly interfere with DNA repair, growth or sporulation. In direct parallel comparisons, the *hrr25::LUK* and *hrr25*Δ alleles behaved identically.

Yeast strain MFH14 (*MATa/MATα ura3/ura3*) was transformed with *Bgl*II-linearized YCp50-*HRR25*::LUK to uracil prototrophy, heterozygous disruption of *HRR25* was verified by Southern blot analysis, the diploid was sporulated by starvation for nitrogen and fermentable carbon sources, tetrads dissected and cells allowed to germinate at 30°C for 7 days. After a normal germination period of 2 days, the severe growth defect of *hrr25*::LUK suggested that the deletion of *HRR25* was lethal. However, microscopic examination of segregants revealed that *hrr25*::LUK germinating cells grew slowly and in every case examined (20/20 tetrads), slow growth, MMS sensitivity, and uracil prototrophy co-segregated. A color variation was seen with diploid MFH14 segregants, due to mutations in adenine biosynthesis. MFH14 is *ade5/ADE5 ade2/ade2*. An *ade5/ade2* strain was white, while an *ADE5/ade2* strain was red.

Example 3

Sequence and Structure of the *HRR25* GENE

DNA sequencing of both strands of the *HRR25* gene was done by uni-directional deletions employing Sequenase (USB, Cleveland, OH) and Exo-Meth (Stratagene, La Jolla, CA) procedures as described by the manufacturers. DNA and deduced amino acid sequences are set out respectively in SEQ. I.D. NOs. 1 and 2. Figure 1A, shows the alignment of the amino acid sequences for *HRR25*, CDC28, KSS1, and RAF1. Figure 1B shows a schematic representation of the structure of *HRR25*. The protein kinase homology is represented by a shaded region while the P/Q rich region is indicated by cross-hatchings. The mutant, *hrr25*, can be distinguished from *HRR25* by one amino acid substitution. At position 151, an aspartic acid is substituted for glycine.

The predicted translation product of *HRR25* revealed an unexpected feature for a *rad*-like DNA repair function. *HRR25* contains the hallmark signatures of sequence homology with the catalytic domain of serine/threonine

protein kinase superfamily members (Hanks, *et al.*, *Science*, 241:42, 1988). For comparison, the *HRR25* translation product was aligned with the catalytic domains for two subgroups of yeast protein kinases, the *CDC28/cdc2* group and the *KSS1/FUS3* group. Located between amino acids 15 and 30 is a region that contains the conserved GXGXXG region. Just C-terminal to this region is a conserved lysine and glutamic acid present in most known kinases. These regions are thought to function in the nucleotide binding and phosphotransfer steps of the kinase reaction (Hanks, *et al.*, *Science*, 241:42,1988). Between amino acid residues 120 to 150 are regions containing the HRD and DFG motifs, also found in most protein kinase family members. In addition, sequence examination of all known serine/threonine kinases indicates that *HRR25* shares some additional similarities with the *Raf/PKS/mos* subgroup (Hanks, *et al.*, *Science*, 241:42, 1988). The strongest homologies can be found in areas around the GXGXXG, DFG, and DXXSXG conserved regions in protein kinase catalytic domains.

The functional relevance of the observed sequence similarity between *HRR25* and protein kinases was studied by altering specific residues within the *HRR25* kinase domain and examining the phenotypic consequences of these changes. A lysine at position 38 (Lys ^{38}) was mutated to an arginine residue by site directed mutagenesis, by methods known in the art. The mutagenic oligonucleotide SEQ. I.D. NO. 22 was:

5'-CCTGATCGATTCCAGCCTGATCGCTACTTCTTCACCACT-3'.

Lys^{38} in *HRR25* corresponds to the lysine found in all known protein kinases, and this subdomain is involved in ATP binding. Mutations at the conserved lysine in protein kinases such as *v-src, v-mos, and DBF2* inactivate these proteins. The mutant *hrr25*-Lys^{38} allele was incapable of complementing *hrr25-1*, *hrr25::LUK*, and *hrr25Δ* alleles for all properties examined, an indication that the *HRR25* kinase domain is required for *in vivo* function of *HRR25*.

The predicted *HRR25* translation product (SEQ. I.D. NO. 2) has a number of notable features outside the region of homology to protein kinase catalytic domains. For example, the last 100 amino acids is proline and glutamine

rich, containing 50 of these residues. Other proteins with regions rich in these two amino acids include the transcription factors *Sp1*, *jun*, and *HAP2*, steroid hormone receptors, the *S. pombe ran1* kinase, and *mak*-male germ cell-associated kinase (Courey, *et al.*, *Cell*, 55:887, 1988; Bohmann, *et al.*, *Science*, 238:1386, 1987; Roussou, *et al.*, *Mol.Cell.Biol.*, 8:2132, 1988; Arriza, *et al.*, *Science*, 237:268, 1987; Matsushime, *et al.*, *Mol.Cell.Biol.*, 10:2261, 1990). In the case of *Sp1* and *jun*, the proline-glutamine regions are involved in transactivation, whereas the P/Q region in the human mineralocorticoid receptor is thought to serve as an intramolecular bridge. This proline-glutamine region in *HRR25* might function as a structural feature for substrate interaction, or for subcellular localization. Also, the glutamine richness of this region is similar to the *opa* or M-repeat seen in the *Drosophila* and *Xenopus Notch/Xotch* proteins (Wharton, *et al.*, *Cell*, 40:55, 1985; Coffman, *et al.*, *Science*, 249:1438, 1990). The function of the *opa* repeat is not certain, but it is found in several *Drosophila* genes. Lastly, the sequence TKKQKY at the C-terminal end of the region homologous to protein kinases is similar to the nuclear localizing signal of SV40 large T antigen and yeast histone H2B (Silver, *et al.*, *J.Cell.Biol.*, 109:983, 1989; Moreland, *et al.*, *Mol.Cell.Biol.*, 7:4048, 1987).

Example 4

Microscopic Analysis of Germinating and Proliferating *hrr25* Cells

Photomicrographs of *HRR25* and *hrr25::LUK* colonies were taken after germination on rich medium. An MFH14 *hrr25::LUK* heterozygous transformant was dissected onto a thin film of YPD rich medium on a sterilized microscope slide and segregants were allowed to germinate under a coverslip by incubating the slide in a moist 30°C chamber. Photographs of colonies were taken after 2 days of growth. Phase contrast and DAPI staining of proliferating *HRR25*Δ and *hrr25::LUK* cells were compared. Cells were inoculated into YPD rich medium and grown at 30°C to a mid-log density of 1-3 X 10^7 cells/ml, briefly sonicated to disrupt clumps, fixed with formaldehyde, and stained with

DAPI (Williamson, *et al., Meth.Cell.Biol.*, 12:335, 1975). Many cells with *hrr25::LUK* lacked DAPI stainable nuclei.

Microscopic examination of germinating and actively growing mid-log phase *hrr25::LUK* cells revealed aberrant cellular morphologies. Transposon disruption of *HRR25* resulted in large cells, and 25-40% of cells were filamentous or extended. DAPI nuclear staining (Williamson, *et al., Meth.Cell.Biol.*, 12:335, 1975) of mid-log populations showed that orderly cell cycle progression in *hrr25* mutants was lost. There were a large number of cells lacking DAPI-stainable nuclei which, by single cell manipulations proved to be inviable. Consistent with this nuclear segregation defect, the plating efficiency of *hrr25::LUK* haploids was also reduced to 75-80% of wild type. However, this reduction in plating efficiency is insufficient to account for the severe growth rate reduction. Plating efficiency was measured from mid-log phase cells by comparing the efficiency of colony formation on rich medium relative to the total number of cells determined by hemocytometer count. Cell populations were analyzed for DNA content distribution by flow cytometric analysis following staining with propidium iodide as described (Hutter, *et al. J.Gen.Microbiol.*, 113:369, 1979). Cell sorting analysis showed that a large number of the cells in a haploid *hrr25::LUK* population were delayed in the cell cycle and exhibited G2 DNA content, but the population was not arrested uniformly in the cell cycle.

Example 5

Sequence Comparison of *HRR25* with *CDC28, KSS1,* and *RAF1*

The predicted translation product of *HRR25* (SEQ. I.D. NO. 2) was compared with the catalytic domains of several members of the serine/threonine protein kinase superfamily. Initial sequence comparisons employed the UWGCG programs (Devereux, *et al., Nuc.Acids.Res.*, 12:387, 1984), whereas subgroup comparisons used the methods of Hanks, *et al., supra*. *HRR25* contains all eleven subdomains described by Hanks, *et al., supra*. Structurally similar groupings were compared in the sequence comparisons. These included nonpolar chain R

groups, aromatic or ring-containing R groups, small R groups with near neutral polarity, acidic R groups, uncharged polar R groups, and basic polar R groups.

CDC28 and *KSS1* represent members of two subgroups of serine/threonine protein kinases in yeast. *CDC28* is involved in cell cycle regulation while *KSS1* acts in the regulation of the yeast mating pathway. *HRR25* shows 21% identity and 41% similarity to *CDC28* and 19% identity and 43% similarity to *KSS1* (Figure 1A). *HRR25* shows highest similarity to members of the *Raf1/PKS/Mos* family of protein kinases. Through the catalytic domain, *HRR25* shows 30% identity and 49% similarity to *Raf1*.

Example 6

Identification, Isolation, and Analysis of *Sc. pombe Hhp1+* and *Hhp2+* Genes

A. Isolation of the *Hhp1+* and *Hhp2+* Genes

The clones were isolated by a two-pronged approach: i) DNA-based screening methods; and ii) direct complementation in *S. cerevisiae hrr25* mutant strains. Two genes were identified (*Hhp1+* and *Hhp2+* - so named for *HRR25* Homologue from *Schizosaccharomyces pombe*). Expression of *Hhp1+* in *S. cerevisiae hrr25* mutants fully rescued all mutant defects. Expression of *Hhp2+* in *S. cerevisiae* also rescued, to varying degrees, the defects associated with *hrr25* mutations.

DNA-based amplification of HRR25-like DNAs from *Sc. pombe* genomic and CDNA sequences prepared according to Fikes, *et al.* (*Nature*, 346:291-293, 1990) was conducted using polymerase chain reaction with the following partially degenerate oligonucleotide primers:

(1) Primer No. 4583 (SEQ. ID. NO. 13) representing top strand DNA encoding residues 16 through 23 of *HRR25*; [1 nmol/5 μ1], T_m = 52°C;

(2) Primer No. 4582 (SEQ. ID. NO. 14) representing top strand DNA encoding residues 126 through 133 of *HRR25*; [1.5 nmol/5 μ1], T_m = 54°C;

(3) Primer No. 4589 (SEQ. ID. NO. 15) representing bottom strand DNA encoding residues 126 through 133 of *HRR25*; [0.5 nmol/5 μ1], T_m = 54°C;

(4) Primer No. 4590 (SEQ. ID. NO. 16) representing bottom strand DNA encoding residues 194 through 199 of *HRR25*; [2 nmol/5 μ1], T_m = 38°C.

Two series of amplifications were conducted using Perkin Elmer Automated apparatus; a first series using *HRR25*-based primer Nos. 4583 and 4589 and a second series employing all four of the primers. In the first series, 30 cycles of denaturation (94°C, 1 min), annealing (48°C, 1 min), and extension (66°C, 3 min) were performed and in a final cycle, the extension time was extended to 5 min. Reaction products were sized on an agarose gel revealing a prominent band of the expected size of about 306 bp. In the second series of amplifications, 30 cycles were carried out as above except that annealing and extension were carried out at 35°C and 60°C, respectively. Three major products of the expected sizes (513 bp, 180 bp, and 306 bp) were developed in both genomic and CDNA libraries and were purified by preparative agarose gel electrophoresis.

Products were cloned into M13mp19 and sequenced by the dideoxy method (Maniatis, *et al.*, *Molecular Cloning: A Laboratory Manual*, 1982). Two classes of sequences were identified. A representative clone from each class was radiolabelled with ^{32}P by random primed cut labeling to a specific activity of 10^6 cpm/μg (Maniatis, *et al.*, *supra*) and used as a hybridization probe to isolate full length CDNA clones and to prove yeast genomic DNA in Southern blots and total RNA on Northern blots. Hybridization was carried out for 16 hours in a buffer

containing 6 x SSPE, 0.1% SDS, 5% dextran sulfate. Two genes were identified and designated Hhp1+ and Hhp2+ for *HRR25* Homologues from *Sc. pombe*.

For *Hhp1+*, 7 clones were identified (6 partial and 1 full length clone). For *Hhp2+*, 2 full length clones were identified. Both Southern and Northern analysis confirmed that these clones were from separate genes. These genes were sequenced using standard dideoxy method (Maniatis, *et al.*, *supra*). The nucleotide and deduced amino acid sequences for *Hhp1+* are set out in SEQ. ID. NOS. 3 and 4; the nucleotide and deduced amino acid sequences for *Hhp2+* are set out in SEQ. ID. NOS. 5 and 6.

B. Functional analysis of *Hhp1+* and *Hhp2+* in *S. cerevisiae hrr25* mutants.

Sc. pombe Hhp1+ and *Hhp2+* cDNAs were cloned in a location which placed them under the control of the *S. cerevisiae* alcohol dehydrogenase-1 (ADH1) promoter in a URA3-based vector pDB20 to allow for expression in *S. cerevisiae* (Fikes, *et al.*, *supra*). These resulting clones were analyzed for their ability to alter/modify the suppress phenotypes associated with the *hrr25-1* mutation and the *hrr25Δ* mutation following transformation into appropriate yeast strains by standard methods (Ito, *et al.*, *J. Bacteriol.* 153:163, 1983). Transformants were analyzed for their ability to overcome defects associated with the *hrr25* mutations (Hoekstra, *et al.*, *Science*, 253:1031, 1991). *Hhp1+* expression fully complemented *hrr25*-associated defects and was indistinguishable from wild type *HRR25* in all analyses. Complementation was analyzed for the effect on DNA repair, cell cycle progression, cellular morphology, and sporulation. *Hhp2+* complemented to a lesser degree than *Hhp1+* (its complementation level was 50%-75% that of *bona fide HRR25*). The alteration of *hrr25*-associated phenotypes was dependent upon the transformed yeast strains containing both a complementing *Sc. pombe Hhp* plasmid and having *hrr25* mutations.

The degree of amino acid homology between *HRR25* protein and *Hhp1+* protein is 73% through the kinase domain. The degree of similarity, which considers the presence of similar as well as identical amino acids, is greater than 85%. The amino acid identity of *HRR25* protein and *Hhp2+* protein is 63%

with a percent similarity score of 80%. The intraspecies comparison of *Hhp1+* protein to *Hhp2+* protein is 72% identity. This structural and complementation analysis clearly indicates that these *Sc. pombe* clones are functional homologues of the *S. cerevisiae HRR25*. Such a high degree of relatedness is not seen with any other group of protein kinases. As a measure of comparison here, *bona fide* functional homologues (i.e., *cdc2* protein kinases from *S. cerevisiae, Sc. pombe*, and humans) show 40%-45% identity. Any two randomly compared protein kinases, regardless of whether the comparison is inter-or intra-species show a degree of identity of about 20%-25%.

C. Disruption and mutation of *Hhp1+* and *Hhp2+* in *Sc. pombe*

Mutations that inactivate or reduce the protein kinase activity of *HRR25* in *S. cerevisiae* result in a wide variety of phenotypes including: sensitivity to various forms of DNA damage, severe cell cycle delay, sensitivity to drugs that affect cell cycle progression (e.g., caffeine), sensitivity to agents that affect microtubule integrity (e.g., benomyl), and sensitivity to agents that affect the integrity of replicating DNA (e.g., hydroxyurea).

Similarity, in *Sc. pombe,* inactivation of the *Hhp1+* and the *Hhp2+* genes to reduce or abolish the encoded protein kinase activity resulted in cellular phenotypes that mimicked *hrr*25 mutations. For example, deletion of the *Hhp1+* gene resulted in a cell cycle delay and aberrant cellular morphology, in sensitivity to DNA damaging agents like MMS, and in sensitivity to benomyl and hydroxyurea. Deletion of the *Hhp2+* gene resulted in caffeine sensitivity, benomyl sensitivity, and hydroxyurea sensitivity, amongst other defects.

The *Hhp1+* gene was disrupted as follows: CDNA was subcloned into the *Sc. pombe* vector pHSS19 (Hoekstra *et al., Meth. Enzymol.*, 194:329, 1991), which was digested with *NheI-EcoRI*. The *Sc. pombe* URA4 gene was inserted resulting in deletion of the *Hhp1+* kinase domain. *Sc. pombe* was transformed by standard methods (Moreno, *et al., Meth. Enzymol.*, 194:795, 1991) with the linearized DNA from the resulting plasmid construction. Stable

transformants were identified and haploid *hhp1*Δ strains were verified by standard methods (Moreno, *et al.*, Maniatis, *et al.*).

The Hhp2+ gene was disrupted as follows: the Hhp2+ CDNA was cloned into the *Sc. pombe* based vector, plasmid pHSS19, and was disrupted by transposon shuttle mutagenesis using the mini-Tn3 transposon mTn3Leu2 (Hoekstra, *et al., Meth. Enzymol supra.*). *Sc. pombe* was transformed by standard methods with the linearized DNA from the resulting plasmid construction. Stable transformants were identified and haploid *hhp2*Δ strains were verified by standard methods (see above).

Standard physiological methods as described for *S. cerevisiae HRR25* (Hoekstra, *et al., Science* 253:1031, 1991) were employed to characterize *hhp* mutant strains. Phenotypic analysis revealed that both *hhp1* and *hhp2* mutants showed defects previously seen in *hrr25* mutants, including sensitivity to various DNA damaging treatments that include MMS treatment and X-ray treatment.

The foregoing substantiates that *Hhp1*+ and *Hhp2*+ are isoforms of *S. cerevisiae HRR25* protein kinase. These three protein kinases show high levels of sequence identity. In addition, mutations that inactivate these kinases result in very similar defects in widely divergent organisms.

D. Complementation of *Sc. pombe* mutant strains with the *S. cerevisiae HRR25* gene.

To show that *Sc. pombe hhp* mutants prepared as described above, were identical to *S. cerevisiae hrr25* mutants and to show that *HRR25*-like protein kinases with greater than 35% amino acid identity are functional homologues, the *S. cerevisiae HRR25* gene was introduced into a *Sc. pombe* expression vector and transformed into *Sc. pombe hhp* mutants. The DNA sequence at the *HRR25* initiating methionine was changed into an *NdeI* site, (a silent coding alteration that maintains the reading frame but allows the *HRR25* gene to be introduced into appropriate *Sc. pombe* plasmids). This was done by a site-directed DNA change was made in the *S. cerevisiae HRR25* gene by standard methods using a commercially available system (Bio-Rad, Cambridge, MA). The altered *HRR25* gene was ligated into the *Sc. pombe* expression plasmid, pREP 1 (Maundrell, K.

J., *Biol. Chem.* 265:10857, 1990), at an *NdeI* site and the resulting construction was transformed by standard methods into *Sc. pombe hhp* mutants. Expression of *HRR25* in *Sc. pombe* mutant strains resulted in complementation of the mutant defects as evaluated by physiological methods described by Hoekstra, *et al.* *(Science, supra).*

Example 7

Isolation and Characterization of Yeast *HRR25*-like Genes

Isolation of additional *HRR25*-like genes from *S. cerevisiae* was accomplished by performing DNA-based amplification of genomic DNA from an *S. cerevisiae* strain lacking *HRR25* coding sequences [Strain 7D of DeMaggio, *et al.* (*Proc. Natl. Acad. Sci., USA*, 89:7008-7012, 1992, incorporated herein by reference) thereby eliminating the chance of obtaining *HRR25* sequences from the amplification. The primers and amplification conditions were as in Example 6.

The resulting amplification products were cloned in M13mp19 and sequenced by dideoxy chain termination methods. Three unique classes of amplified products were identified. Two of these products respectively corresponded to the YCK1/CKI2 and YCK2/CKI1 genes of Robinson, *et al.* (*Proc. Natl. Acad. Sci. USA*, 89:28-32, 1992) and Wang, *et al.* (*Molecular Biology of the Cell*, 3:275-286, 1992). The third gene product was designated "NUF1" (for Number Four). The amplified products corresponding to NUF1 were radiolabelled as described in Example 6 and used to screen a yeast YCp50-based genomic library (ATCC, Rockville, MD). Eight clones were identified and one of these clones included approximately 4 Kb *Hind*III fragment containing the NUF1 hybridizing gene. Southern analysis revealed that NUF1 is a separate gene from *HRR25*, YCK1/CKI2, and YCK2/CKI1. The *Hind*III fragment was sequenced and revealed a protein kinase with about 65% identity to *HRR25* through its protein kinase domain. The DNA and deduced amino acid sequences for NUF1 are set out in SEQ. ID. NOS. 23 and 24.

To further characterize the NUF1 gene, the *Hind*III fragment was subcloned into the yeast plasmid YEplac112 [Gietz and Sugino, *Gene 74*:527-541

(1988)]. The resulting construct was transformed into the hrr25Δ deletion strain 7d and NUF1 was found to complement for hrr25Δ mitotic defects (e.g., NUF1 complemented for slow growth defect, aberrant morphology defect, DNA damaging agent sensitivities). Furthermore, a null mutant allele of NUF1 was constructed by transposon shuttle mutagenesis and strains lacking the NUF1 gene product were found to have hrr25Δ mutant-like defects. In particular, like hrr25Δ mutants, NUF1 mutants showed slower mitotic growth rates and increased sensitivity to DNA damaging agents like MMS, UV, and X-irradiation.

Example 8

Identification and Isolation of

Human *HRR25*-like Genes

Oligonucleotides derived from amino acid sequences described above in Example 6A were used to amplify cDNAS from the following sources: *Arabidopsis thaliana, Drosophila melanogaster, Xenopus*, chicken, mouse, rat, and human HeLa cells. These cDNAS were obtained from reverse transcribed mRNA (Maniatis, *et al., supra*) or from commercially-available cDNA libraries (Stratagene, La Jolla, CA, and Clonetech, Palo Alto, CA) Amplification products of similar migration size to those obtained from *S. cerevisiae HRR25* and *Sc. pombe*, *Hhp1+* and *Hhp2+* genes were observed in 1.0% Agarose gels (Maniatis, *et al., supra*). This result indicated that *HRR25*-like genes exist in all species examined.

Isolation of full length DNAs encoding human *HRR25*-like protein kinases was accomplished by PCR amplification of human genomic DNA using unique sequence oligonucleotide primers based on portions of a bovine brain casein kinase I cDNA which had been reported in Rowles, *et al.* (*Proc. Natl. Acad. Sci. USA*, 88:9548-9552, 1991) to encode a mammalian protein that was 60% homologous to *HRR25* over its catalytic domain.

A variety of primers were prepared and used in pairwise fashion including:

(1) Primer JH21 (SEQ. ID. NO. 17) representing bovine top strand DNA bases 47-67;

(2) Primer JH22 (SEQ. ID. NO. 18) representing bovine top strand DNA bases 223-240;

(3) Primer JH29 (SEQ. ID. NO. 19) representing bovine top strand DNA bases 604-623;

(4) Primer JH30 (SEQ. ID. NO. 20) representing bovine top strand DNA bases 623-604; and

(5) Primer JH31 (SEQ. ID. NO. 21) representing bovine top strand DNA bases 835-817.

DNA amplification with combination of oligonucleotides JH21/JH30, JH22/JH30, and JH29/JH31 were carried out for 30 cycles with denaturation performed at 94°C for 4 min for the first cycle and for 1 min for the remaining cycle annealing at 50°C for 2 min and extension at 72°C for 4 min. Products of the expected size from the three amplifications were purified on preparative acrylamide gels and labeled with ^{32}P using random nick translation (to a specific activity between 7 x 10^6 cpm/μg and 1.4 x 10^7 cpm/μg. The labelled probes were employed as a group to screen a commercial human fetal brain cDNA library (Stratagene). Hybridization was carried out for 16 hours at 65°C in a hybridization buffer containing 3 x SSC, 0.1% Sarkosyl, 10 x Denhart's solution and 20 mM sodium phosphate (Ph 6.8). Three washes at 65°C in 2 x SSC, 0.1% SDS were performed. Approximately 1.5 x 10^6 plaques were screened on 30 plates using duplicate filters. Six strong positive clones were isolated, purified and converted to plasmid form according to procedures recommended by the supplier of the library. Restriction digestion revealed the

following insert sizes for the six clones: clone 35A1, 1kb; clone 35B1, 1.4kb; clone 41A1, 3.7kb; clone 42A1, >4kb; clone 47A1, 3.35kb; and clone 51A1, 2.75kb. All six inserts contained sequences which could be aligned with both the DNAs and deduced protein sequence of the bovine CKIα gene. The abbreviated, partial cDNA clones 35A1 and 35B1 were not further analyzed. Clones 41A1 and 42A1 were identical except for size. Clones 42A1, 51A1, and 47A1 were redesignated as CKIα1Hu, CKIα2Hu, and CKIα3Hu. The DNA and deduced amino acid sequences of the inserts are set out in SEQ. ID. NOS. 7 and 8; 9 and 10; and 11 and 12, respectively. The deduced amino acid sequence for CKIα1Hu was identical to the reported bovine CKIα sequence. Table 1, below sets out differences in nucleotides between the bovine and human DNAs, numbered from the first base in the initiation codon, ATG.

TABLE 1

COMPARISON OF HUMAN AND BOVINE CKIα DNA

Position	Bovine	Human	Position	Bovine	Human
+ 9	C	T	+591	A	G
+ 27	A	T	+594	A	G
+ 93	T	C	+669	A	G
+126	G	A	+687	A	G
+147	C	T	+690	G	A
+186	A	G	+705	A	G
+255	T	C	+729	A	G
+258	C	T	+731	C	T
+261	G	A	+753	A	G
+267	T	C	+771	C	G
+279	T	G	+798	G	A
+285	C	T	+816	G	A
+291	T	C	+828	C	T
+372	C	T	+867	T	C
+540	T	C	+870	C	T
+555	T	C	+936	A	C
+558	G	A			

The CKIα3Hu DNA also includes an insertion of 84 bases at position +454 in the coding sequence providing an intermediate extension of the CKIα2Hu expression product by 28 amino acids. This DNA insert is not present in the bovine gene, but it encodes an amino acid sequence insert which Rowles, *et al.* designated as CKI-alpha-L. The CKIα2Hu and CKIα3Hu DNAs insertion at position +971 of the CKIα1Hu DNA. This insertion is not found in any of the bovine sequences and encodes an extension of the 13 amino acids adjacent the carboxy terminal. The last two codons of the CKIα3Hu sequences differ from

any of the bovine sequences or the sequences of CKIα1Hu and CKIα2Hu, causing the CKIα3Hu expression product to terminate with a lysine, rather than a phenylalanine as found in all the other bovine and human casein kinase I sequences. The 3' flanking sequence of CKIα3Hu DNA differs significantly from that of CKIα1Hu and CKIα2Hu.

FIGURE 2 provides an alignment of the catalytic domain amino acid sequences of *HRR25*-like proteins whose DNAs were isolated in the above illustrative examples, including *HRR25*, *Hhp*1+, *Hhp*2+, CKIα1Hu, CKIα2Hu, and CKIα3Hu as well as YCK1/CKI2, and YCK2/CKI1. Note that with the exception of the CKIα3Hu intermediate insert and the carboxy terminal region inserts of CKIα2Hu and CKIα3Hu, the sequences of the three human products are identical. "Common" residues are indicated in the Figure where at least 3 of the seven residues are identical at the corresponding position (the human sequences being taken as a single sequence).

Like *Hhp1*+ and *Hhp2*+, the three human *HRR25*-like protein kinases showed very high degrees of amino acid identity to the *HRR25* gene product (68%), establishing that these human clones were enzymatic isoforms of the yeast *HRR25* gene. The alignment of *HRR25*, *Hhp1*+, *Hhp2*+, and the human complementing-like kinase isoforms show that these enzymes share a number of primary structural features that indicate that these enzymes provide comparable activities in different species. This conclusion is reached based on several lines of evidence. First, all enzymes share the common primary sequence identifiers characteristic of protein kinases. Second, the enzymes share high degrees of amino acid identity in regions of the protein kinase domain that are not conserved in unrelated protein kinases. Finally, these enzymes share regions of identity in the kinase domain which regions differ in primary sequence from other protein kinases, but are identical among the members of this isoform grouping. For example, greater than 95% of all known protein kinases have a so-called A-P-E sequence (Alanine-Proline-Glutamate) approximately two-thirds of the way through the kinase domain. *HRR25*-like protein kinases lack the A-P-E sequence and have instead a S-I/V-N sequence (Serine-Isoleucine or Valine-Asparagine). Based on this primary sequence comparison, between known protein kinases and

the protein kinases of the invention from evolutionarily divergent organisms, these enzymes of the invention are isoforms of *HRR25* protein kinase.

Example 9

Comparison of *HRR25* with a Casein Kinase

In all eukaryotes examined, two of the major protein kinases are casein kinase I and II (CKI and CKII, respectively). These enzymes have been found in all cell types and species examined. Both enzymes recognize Ser/Thr residues in an acidic environment in the substrate. These two protein kinases are found throughout the cell and their activities have been purified from or found to be associated with cytoplasmic fractions, membranes, nuclei, mitochondria, and cytoskeleton. CKII is predominantly a nuclear enzyme, but similar studies have yet to be described for CKI.

To determine whether *HRR25* gene product might function as a casein kinase, the ability of *HRR25*-containing immunoprecipitates to phosphorylate casein was studied. *HRR25*-containing immunoprecipitates from yeast were incubated with casein and phosphorylated proteins were examined.

Yeast extracts were prepared by physical disruption. Equal volumes of a cells were suspended in lysis buffer and acid-washed 0.5 mm beads were mixed, 30 second bursts were interspersed with 1 min on ice, and the extent of disruption was followed microscopically. Lysis buffer contained 10 Mm sodium phosphate (Ph 7.2), 150 Mm NaCl, 1% Nonidet P-40, 1% Trasylol, 1 Mm DTT, 1 Mm benzamidine, 1 Mm phenylmethyl sulfonyl fluoride, 5 Mm EDTA, pepstatin (1 ug/ml), Pepstatin A (2 ug/ml), leupeptin (1 ug/ml), 100mM sodium vanadate, and 50 Mm NaF. Extracts were clarified by a 100,000 x g centrifugation for 30 min., made to 50% (vol/vol) with glycerol, frozen in liquid nitrogen, and stored at -70 degrees C. Little loss in protein kinase activity was seen in frozen extracts over several months.

Immune complex protein kinase assays were performed on the extracts according to the methods described in Lindberg, *et al. (Mol. Cell. Biol.* 10:6316, 1991). Frozen extracts were diluted to 25% glycerol with lysis buffer

or fresh extracts were used directly. Extracts were precleared with preimmune serum and protein A-Sepharose, and then treated with immune serum (obtained as described in Example 11, *infra*, from immunization of rabbits with *E. coli*-derived type-*HRR25* fusion products). *HRR25* kinase-containing immune complexes were precipitated with protein A-Sepharose. Immune complexes were washed four times with lysis buffer and twice with kinase buffer containing 15 Mm Hepes (Ph 7.4), 100 Mm NaCl, and 10 Mm $MgCl_2$.

Reaction mixtures of *HRR25* immunoprecipitates and heat-treated casein (300 ng/20ul reaction volume) were incubated at 30 degrees C for 5-10 min and contained 10 uCi of gamma-^{32}P-ATP per 20 ul reaction volume. Reactions were stopped by the addition of SDS and EDTA, boiled in SDS/PAGE sample buffer and resolved in 10 % gels. Phosphoamino acid analysis was as described (Hunter *et al., Proc.Natl.Acad.Sci.USA* 77:1311, 1980).

Immunoprecipitates from *HRR+* strains were able to phosphorylate casein. To verify that the appropriate amino acids were phosphorylated, the phosphoamino acid composition of the *HRR25*-phosphorylated casein was examined by phosphoamino acid analysis. Samples were resolved by two-dimensional electrophoresis at Ph 1.9 and Ph 3.5. Consistent with mammalian CKI specificity, serine and threonine residues were phosphorylated. *HRR25* phosphorylated serine residues on casein 3-fold greater than threonine residues. Similarly, the autophosphorylation of *HRR25* in immune complexes *in vitro* occurred on serine and threonine residues. Coupled with the high degree of sequence identity, these results suggest that *HRR25* might be a CKI isoform.

To extend and confirm that *HRR25* immunoprecipitates from yeast could phosphorylate casein, several experiments were performed. *HRR25* immunoprecipitated from *E. coli* strains expressing *HRR25* (See Example 11) also showed casein kinase activity, whereas *E. coli* extracts lacking *HRR25* protein did not phosphorylate casein. *HRR25*-containing baculovirus constructs produced casein kinase activity in immunoprecipitates. Wild-type baculovirus-infected cells showed ⟨0.5% casein kinase activity under comparable conditions. The protein kinase activity from S19 cells expressing *HRR25* protein was sensitive to the same conditions that reduced or inactivated the *HRR25* protein activity from yeast

extracts. The observations that *HRR25*-dependent casein kinase activity was present in immunoprecipitates from *E. coli* cells expressing wild-type *HRR25*, in insect cells infected with *HRR25*-containing baculovirus, and in wild-type but not *hrr25*$_\Delta$ mutants indicated that the *HRR25* gene product could function as a casein kinase and that the casein kinase activity in *HRR25* protein-containing immunoprecipitates was due to *HRR25* gene product.

Example 10

Analysis of Protein Kinase Activity of *HRR25*-like Proteins

Because the predominant protein kinase activity in *E. coli* is histidine kinase, rather than serine/threonine or tyrosine kinase, those procaryotic cells provide a system for examination of *HRR25*-like protein kinase activities which is not compromised by presence of endogenous kinases. Both *HRR25* and Hhp1 + DNAs were, therefore, expressed in the IPTG-inducible T7 gene 10-based commercial expression system (Invitrogen, San Diego, CA) using *E. coli* strain BL21 (DE3) which contains an IPTG-inducible T7 RNA polymerase and T7 lysozyme gene. See, DeMaggio, *et al., Proc. Natl. Acad. Sci. USA,* 89:7008-7012, (1991). In a first series of experiments, *E. coli* lysates were prepared by inducing mid-log phase cells with IPTG for 2 hours, pelleting the cells, and preparing extracts by a freeze-thaw method using buffers described in DeMaggio, *et al., supra*. Extracts were electrophoresed in polyacrylamide gels, transferred to nylon-based support membranes, and probed by Western analysis with antibodies directed against phosphotyrosine (UBI, Lake Placid, NY). These procedures revealed that *HRR25* and *Hhp1+* expressing cells contained novel tyrosine phosphorylated proteins not observed in control cells (transformed with the vector alone or with kinase inactive mutants). In a second experiment, the *HRR25* and *Hhp1+*-containing *E. coli* strains were examined for tyrosine-phosphorylated protein by a sensitive and accurate radiolabelling and phosphoamino acid procedure. To do this experiment, cells were induced with IPTG and grown in the presence of ^{32}P-orthophosphate. Radiolabelled extracts were prepared by the freeze-thaw method, electrophoresed in polyacrylamide gels,

and the gels were examined by autoradiographic methods. Novel phosphoproteins were observed in the strains expressing *HRR25* and *Hhp1+*, but not in the above controls. Phosphoproteins were examined by extracting and hydrolyzing the proteins from the gels using standard methods (Boyle, *et al.*, *Meth. Enzymol*, 201:110, 1991). These experiments verified that *HRR25* and *Hhp1+* could phosphorylate tyrosine, serine, and threonine residues on protein substrates.

Example 11

Recombinant Expression of HRR25

Products and Generation of Antibodies Thereto

Two different plasmid constructions were developed for expression of HRR25 DNA in *E. coli* to generate immunogens useful in preparation of anti-HRR25 antibodies.

The first plasmid construction involved plasmid pATH according to Koerner *et al.*, *Meth. Enzymol.*, 194:477-491 (1991). An approximately [606] base pair DNA fragment was isolated from the HRR25 open reading frame by *Bgl* II digestion and this fragment (which encodes amino acid residues 275-476) was ligated into pATH which had been digested with *Bam*HI. The resulting plasmid encoded a fusion protein comprising the *E. coli* TrpE gene product at its amino terminus and a carboxy terminal fragment of HRR25 at its carboxyl terminus.

Inclusion bodies were isolated from *E. coli* DH5α (Bethesda Research Laboratories, Bethesda, MD) host cells transformed the plasmid using lysis buffers as described in Koerner *et al.*, *supra*, and were purified by polyacrylamide gel electrophoresis. The gel purified materials were then employed in the immunization of rabbits by subcutaneous injection as recommended by Harlow, *et al.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1988), using gel purified products with complete Freund's adjuvant for primary injections and incomplete Freund's adjuvant for subsequent injections. Serum reactivity was followed by Western blotting against the gel purified antigen. Affinity purification of serum antibodies was effected using the *E. coli*-produced antigen immobilized on a nitrocellulose membrane support.

Example 12

Isolation of CKI-γ1Hu and CKI-γ2Hu

Additional human HRR25-like protein kinase encoding DNAs were isolated by combined DNA amplification and library screening methods. Oligonucleotides based on conserved regions in HRR25-like protein kinases were used to amplify DNA segments for use as probes in screening human a cDNA library. Redundant oligonucleotides of the sequence

5′-GAR YTI MGI YTI GGI AAY YTI TA-3′ (SEQ ID NO. 28)

and

5′-GTY TTR TTI CCI GGI CKI CCI AT-3′ (SEQ ID NO. 29)

(where G, A, T, and C = standard nucleotides and R = A and G; Y = C and T; I = Inosine; M = A and C; and K = G and T) were used to amplify an approximately 540 nucleotide from a human fetal brain cDNA library (Clonetech). Amplification conditions used 200 Mm Tris.Hcl (Ph 8.2), 100 Mm KCl, 60 Mm (NH4)2SO4, 15 Mm MgCl2, 1% Triton X-100, 0.5 μM of each primer, 100 ng library DNA template, 200 μM dNTPs and 2.5 U polymerase. The reactions were performed for 30 cycles. Reactions were started with a 4 minute treatment at 94°C and all cycles were 1 minute at 94°C, 2 minutes at 5°C for annealing, and 4 minutes at 72°C for extension.

The amplification reaction was electrophoresed through a 1% agarose gel and the region corresponding to approximately 540 base pairs was excised and DNA was eluted using a NaI extraction and glass powder binding (GeneClean, Bio101, La Jolla, CA). The gel-purified fragment was ligated into SmaI-digested Bluescript II SK(+) and the resulting plasmid contained a partial protein kinase domain that was used as a source of cDNA for library screening. Ten micrograms of this plasmid was digested with *Eco*RI and *Bam*HI to liberate the subcloned fragment and the reaction was electrophoresed through a 1% agarose gel. The approximately 540 nucleotide fragment was eluted from the gel and was radiolabelled by random primed oligonucleotide directed labelling (Amersham, Arlington Heights, IL) using ^{32}P-dCTP as the radioactive nucleotide.

The radioactive probe was used to screen a human Manca B cell lymphoma library [Wiman, *et al.*, *Proc.Natl.Acad.Sci. (USA) 81*:6798-6802 (1984)] prepared in phage cloning vector λgt10 prepared as follows. Poly d(A)$^+$RNA was prepared from 2.8 x 10^8 cells of the B-cell lymphoma Manca using the "Fast Track" kit (Invitrogen). 5 μg of RNA was used for oligo d(T) primed cDNA synthesis with the cDNA Synthesis System (Gibco BRL, Burlington, Ontario, Canada); the resulting cDNA was size selected by agarose gel electrophoresis and ligated to *Eco*RI adapters with the Ribo Clone kit (Promega, Madison, WI). Varying amounts of the adapted cDNA were ligated to *Eco*RI-digested λgt10 with 1 unit of T4 DNA ligase (Boehringer Mannheim, Indianapolis, IN) in a commercially prepared buffer supplied by the manufacturer with the enzyme. The ligations were packaged with Gigapack packaging extracts (Stratagene) and the resulting phage pool (1.5 x 10^6 phage) was amplified in the C600 Hfl strain. A total of 1 x 10^6 phage plaques were screened by standard hybridization methods (Maniatis, *et al.*, *supra*). Hybridizations were at 65°C for 18 hours in 6X SSPE (20X SSPE is 175.3 g/l NaCl, 27.6 g/l $NaH_2PO_4.H_2O$), 7.4 g/l EDTA, pH 7.4), 100 μg/ml salmon sperm carrier DNA, 5X Denhardt Reagent (50X Denhardts is 5% ficoll, 5% polyvinyl pyrolidone, 5% bovine serum albumin), 0.1% SDS and 5% sodium dextran sulfate. Filters were washed four times in 0.1X SSPE, 1% SDS. Each wash was at 65°C for 30 minutes. Five clones were chosen for further analysis.

DNA from these phage clones was prepared using a Qiagen lambda DNA preparation kit (Qiagen, Chatsworth, CA) and human cDNA inserts were excised by *Eco*RI digestion. These inserts were subcloned into *Eco*RI-digested plasmid Bluescript II SK(+) (Stratagene) and the inserts were sequenced using an ABI 373A automated DNA sequencer. Two of the five cDNA contained near full-length cDNAS with a polyA tail and a protein kinase open reading frame. These protein kinases were most closely related to isoforms of casein kinase I were designated CKIγ1Hu and CKIγ2Hu. The DNA sequences of CKIγ1Hu and CKIγ2Hu are set out in SEQ ID NOS: 30 and 32, respectively; the deduced amino acid sequences of CKIγ1Hu and CKIγ2Hu are set out in SEQ ID NOS: 31 and 33, respectively.

Example 13

Isolation of CKIδHu

Human CKIδ was subcloned by first isolating the human gene from a human fetal brain library constructed in λZAPII (Stratagene). A 2.2 kb *Eco*RI fragment containing rat CKIδ was gel purified through 1% agarose, isolated from the gel by NaI extraction with glass powder (Bio101, La Jolla, CA), and radiolabelled by random primer methods (Boehringer Mannheim) using ^{32}P-dCTP. This probe was used to screen 1 X 10^6 plaques containing human fetal brain cDNA library. Plaque hybridization conditions were 3X SSC, 0.1% Sarkosyl, 10X Denhardts reagent, 50 μg/ml salmon sperm DNA carrier. Hybridization was allowed to proceed for 18 hours at 65°C after which time the filters were washed 4 times for 30 minutes each at 65°C in 2X SSC, 1.0% SDS. Positive clones were identified by autoradiography at -70°C with an enhancing screen and sequenced using an automated ABI373A DNA sequencer (Applied Biosystems, Foster City, California)..

One clone was determined to encode a full length CKIδ isoform and was designated CKIδHu. The nucleotide sequence for CKIδHu is set out in SEQ ID NO: 34, and the deduced amino acid sequence is set out in SEQ ID NO: 35.

Expression of the CKIδHu isoform was then determined in eight different human tissues using an approximately 1.2 kb *Eco*RI fragment as a probe. CKIδHu mRNA levels were highest in kidney, liver and placenta cells, in contrast to the testes-specific expression of rat CKIδ demonstrated by Graves, *et al.*,[*supra*].

Table 2 - Sequence Homology Between CKI Isoforms

	HRR25	Human CKIα1	Human CKIγ1	Human CKIγ2	Human CKIδ
HRR25	100	68	50	50	65
Human CKIα1		100	52	52	76
Human CKIγ1			100	99	55
Human CKIγ2				100	55
Human CKIδ					100

Example 14

Complementation of Yeast CKI Mutants by Human CKI Genes

In order to determine if CKIγ1Hu was an isoform of yeast HRR25-like protein the gene was expressed in yeast protein kinase mutants. The cDNA was expressed under control of the yeast GAL1 promoter. The expression plasmid was a derivative of plasmid pRS305 (Stratagene) that contains the yeast GAL1 promoter. The parental plasmid with the GAL1 promoter was previously described [Davis *et al.*, *Cell 61*:965-978 (1990)] and contained a *Bgl*II site adjacent to the GAL1 promoter as well as *Bam*HI and *Sac*I sites adjacent to the *Bgl*II site. This plasmid was modified by site-directed mutagenesis to contain a unique *Nco*I site between the GAL1 promoter and the *Bgl*II site. The *Nco*I site was adjacent to the GAL1 promoter such that the order of genetic elements was GAL1 promoter-*Nco*I-*Bgl*II-*Bam*HI-*Sac*I. Site-directed mutagenesis (MutaGene kit, BioRad) employed the oligonucleotide

5′-CTA GAT CTA GCT AGA CCA TGG TAG TTT TTT CTC CTT GAC G-3′ (SEQ ID NO. 36)

and generated a unique *Nco*I site (underlined in SEQ ID NO: 36). The resulting plasmid was called pRS305(N) 2μ GAL1.

To clone CKIγ1Hu into pRS305(N) 2μ GAL1, the CKIγ1Hu cDNA was amplified from cDNA with oligonucleotides that would introduce an *Nco*I site at the initiating ATG and a *Bam*HI site in the 3' untranslated region. The sequence of the mutagenic oligonucleotide (with the *Nco*I site underlined) for the amino terminus was

5'-CAT GCC ATG GCA CGA CCT AGT-3' (SEQ ID NO: 37).

The oligonucleotide M13rev, purchased from Stratagene (Stratagene, La Jolla, CA) was used to introduce the *Bam*HI site in the 3' untranslated region. Amplification conditions used 200 Mm Tris-HCl (Ph 8.2), 100 Mm KCl, 60 mM $(NH_4)_2SO_4$, 15 mM $MgCl_2$, 1% Triton X-100, 0.5 μM of each primer, 100 ng template, 200 μM of each dNTP and 2.5 units polymerase. The reactions were performed for 30 cycles. Reactions were started with a 4 minute treatment at 94°C and all cycles were 1 minute at 94°C for denaturing, 2 minutes at 50°C for annealing, and 4 minutes at 72°C for extension. The amplified product was digested with *Nco*I and *Bam*HI and was cloned into *Nco*I/*Bam*HI-digested pRS305(N) 2μ GAL1.

Complementation of yeast CKI mutants employed yeast strains 7D (*hrr 25 Δ, ura3-1, trp1-1, leu2-3, 112, his3-11,15, can1-100, ade2-1*) [DeMaggio, *et al., (1992) supra*] and YI227 (*cki1D, cki2D, FOA^R, ade2-1, can1-100, his3-11,15, leu2-3,12, trp1-1, ura3-1, pRS415::Cki1ts*) Strain 7D lacked the HRR25 isoform of yeast CKI and strain YI227 contained a temperature sensitive allele of yeast CKI1. Yeast strains were transformed by lithium acetate-mediated transformation methods and transformants were selected on SD-leucine medium (Bio101). Controls for transformation were plasmids pRS305(N) 2μg GAL1 alone, plasmid pRS315 (Stratagene), and plasmid pRS315::HRR25, which contains a SalI-EcoRI genomic fragment that spans the genomic HRR25 fragment [Hoekstra *et al., Science, supra*]. Plasmid pRS315::HRR25 was constructed by ligating a *Sal*I/*Eco*RI genomic fragment of HRR25 into *Sal*I/*Eco*RI-digested

pRS315. Both HRR25 and CKIγ1Hu, when expressed in yeast mutants, are capable of fully complementing for the temperature-sensitive growth defect of CKI. In addition, CKIγ1Hu partially suppressed a severe growth rate defect associated with HRR25 mutants. The partial suppression of HRR25 growth defects by CKIγ1Hu was detected by a 10-20 fold greater plating efficiency relative to pRS305(N) 2μ GAL1.

To extend the complementation analysis to additional CKI family members, the ability of other human CKIαHu and CKIδHu genes to complement for the HRR25 mutant defects was examined. Human CKIα1Hu was subcloned into plasmid pRS305(N) 2μ GAL1 by first introducing an *Nco*I site at the initiating methionine by site-directed mutagenesis. The mutagenic oligonucleotide (with the *Nco*I site underlined) was

5′-CTA GAT CTA GCT AGA CCA TGG TAG TTT TTT CTC CTT GAC G-3′
(SEQ ID NO. 38)

and mutagenesis was performed using the Mutagene kit (BioRad). The mutagenized cDNA was digested with *Nco*I and *Bgl*II and the CKIα1Hu fragment was ligated into pRS305(n) 2μ GAL1.

Two constructs containing the CKIδHu cDNA were examined for complementation. Plasmid pEC7B (containing CKIδHu cDNA) was used as a template for site-directed mutagenesis (MutaGene, BioRad). The mutagenic oligonucleotide

5′-GAA TCG GGC CGC CGA GAT CT*C ATA TG*G AGC TGA GAG TC-3′
(SEQ ID NO: 39)

was used to introduce *Bgl*II (underlined in SEQ ID NO: 39) and *Nde*I (in italics in SEQ ID NO: 39) sites at the initiating ATG of CKIδHu. One plasmid construction employed *Bgl*II/*Sac*I-digested CKI DNA from the mutagenized cDNA that was ligated into *Bgl*II/*Sac*I-digested pRS305(N) 2μ GAL1 to produce

pRS305(CKIδ). The second plasmid construct employed *Nco*I/*Sac*I-digested CKIδHu cDNA from unmutagenized pEC7B cDNA that was ligated into *Nco*I/*Sac*I-digested pRS305(N) 2μ GAL1 to produce pRS305(N)(CKIδ). Plasmid pRS305(CKIδ) contained the nucleotides

5′-CCC GGA TCT AGC AGA TCT CAT-3′ (SEQ ID NO: 40)

between the GAL1 promoter and the initiating methionine of CKIδ. Plasmid pRS305(N)(CKIδ) had a near-perfect fusion between the initiating methionine of CKIδHu and the 3' end of GAL1. Near perfect fusion indicates that the promoter and initiating methionine codon have few or no intervening nucleic acid sequences, and therefore are approximately abutting.

The CKIα1Hu and CKIδHu-containing plasmids were transformed into yeast strains 7D and YI227 and were examined for their ability to complement for their mutant defects. Like CKIγHu, CKIα1Hu partially complemented the growth defect associated with HRR25 mutations. CKIδHu was able to complement for the growth defect of temperature-conditional CKI strains, for the growth defect of HRR25 mutants, and for the DNA repair defect of HRR25. The ability of CKIδHu to complement for mutant defects in these yeast strains was indistinguishable from yeast HRR25 or CKI genes only when the appropriate plasmid construct was employed. Plasmid pRS305(CKIδ), which contained the additional 21 bases was unable to complement for any mutant phenotypes, while the near-perfect fusion in pRS305(N)(CKIδ) was fully functional. This difference was attributed to the inability of yeast to translate extended and/or CG rich leader sequences.

Example 15

Generation of Monoclonal Antibodies

A. CKIαHu Peptides

Monoclonal antibodies were raised against the following peptides. SEQ ID NO: 41 was derived from the common amino terminus of CKIα1Hu, CKIα2Hu, and CKIα3Hu, and SEQ ID NO: 42 was derived from an internal alternative splice region in CKIα3Hu.

NH_2-ASSSGSKAEFIVGGY-COOH (SEQ ID NO: 41)
NH_2-RSMTVSTSQDPSFSGY-COOH (SEQ ID NO: 42)

These peptides were initially each coupled to bovine gamma globulin (Sigma, St Louis, MO). Five mg of gamma globulin and 5 mg of peptide were resuspended in 0.4 ml 100 mM K_2HPO_4 (pH 7.2) and to this mixture, 35 mg 1-ethyl-3(3-dimethylamino propyl)-carbodiimide-HCl (EDC, Pierce) previously dissolved in 50 μl K_2HPO_4 (pH 7.2) was added. The reaction was allowed to proceed for 16 hr at 4°C and was quenched by addition of 0.25 ml 2 M ethanolamine and 0.25 ml acetic acid. The reaction mixture was then diluted to a final volume of 2.5 ml with PBS and desalted using Sephadex G-25M (Pharmacia) chromatography. Protein containing fractions were concentrated by centrifugal microconcentration (Amicon). Mice were then injected with 50 μg of the coupled peptide nine times over a period of 8 months. Antibody production was measured against the respective peptides by ELISA.

Fusions were performed by standard methods. Briefly, a single-cell suspension was formed by grinding the spleen between the frosted ends of two glass microscope slides submerged in serum free RPMI 1640 media, supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 μg/ml streptomycin (RPMI) (Gibco). The cell suspension was filtered through a sterile 70-mesh Nitex cell strainer (Becton Dickinson, Parsippany, NJ), and washed twice by centrifuging at 200 g for 5 minutes and the pellet resuspended in 20 ml serum free RPMI. Thymocytes taken from 3 naive Balb/c mice were prepared in a similar manner.

NS-1 myeloma cells, kept in log phase in RPMI with 11% fetal bovine serum (FBS) (Hyclone, Laboratories, Inc., Logan,Utah) for three days prior to fusion, were centrifuged at 200 g for 5 minutes, and the pellet was washed twice as described in the foregoing paragraph. After washing, each cell suspension was brought to a final volume of 10 ml in serum free RPMI, and 10 μl was diluted 1:100. From each dilution, 20 μl was removed, mixed with 20 μl 0.4% trypan blue stain in 0.85% saline (Gibco), loaded onto a hemacytometer (Baxter Healthcare Corp., Deerfield, IL) and cells counted.

Approximately 2 x 10^8 spleen cells were combined with 4 x 10^7 NS-1 cells, centrifuged, and the supernatant was aspirated. The cell pellet was dislodged by tapping the tube and 2 ml of 37°C PEG 1500 (50% in 75 mM Hepes, Ph 8.0) (Boehringer Mannheim) was added with stirring over the course of 1 minute, followed by adding 14 ml of serum free RPMI over 7 minutes. An additional 16 ml RPMI was added and the cells were centrifuged at 200 g for 10 minutes. After discarding the supernatant, the pellet was resuspended in 200 ml RPMI containing 15% FBS, 100 μM sodium hypoxanthine, 0.4 μM aminopterin, 16 μM thymidine (HAT) (Gibco), 25 units/ml IL-6 (Mallinckrodt, St. Louis, MO) and 1.5 x 10^6 thymocytes/ml. The suspension was dispensed into ten 96-well flat bottom tissue culture plates (Corning, Essex, United Kingdom) at 200 μl/well. Cells in the plates were fed 2-3 times between fusing and screening by aspirating approximately half of the medium from each well with an 18 gauge needle (Becton Dickinson), and replenishing plating medium described above except containing 10 units/ml IL-6 and lacking thymocytes.

Fusions were screened when cell growth reached 60-80% confluency (usually 7-9 days). Fusion 75 was screened by ELISA on either the common amino terminal peptide (SEQ ID NO: 41) or the internal peptide (SEQ ID NO: 42), and fusion 80 was screened on the amino terminal peptide (SEQ ID NO: 41) only. Immulon 4 plates (Dynatech, Cambridge, MA) were coated at 4°C overnight with 100 ng/well peptide in 50 mM carbonate buffer, Ph 9.6. Plates were washed three times with PBS containing 0.05% Tween 20 (PBST) and 50 μl culture supernatant was added. After incubation at 37°C for 30 minutes, and washing as above, 50 μl horseradish peroxidase conjugated goat anti-mouse

IgG(fc) (Jackson ImmunoResearch, West Grove, PA) diluted 1:3500 in PBST was added. Plates were incubated as above, washed four times with PBST and 100 μl substrate, consisting of 1 mg/ml *o*-phenylene diamine (Sigma) and 0.1 μl/ml 30% H_2O_2 in 100 mM citrate, pH 4.5, was added. The color reaction was stopped in 5 minutes with the addition of 50 μl of 15% H_2SO_4. Absorbance at 490 nm was read on a plate reader (Dynatech).

Three wells from each fusion (designated 75D3G, 75C10H, 75C2g, 80G10H, 80H4F, and 80J9E) were cloned two to three times, successively, by doubling dilution in RPMI, 15% FBS, 100μM sodium hypoxanthine, 16 μM thymidine and 10 units/ml IL-6. Wells of clone plates were scored visually after 4 days and the number of colonies in the least dense wells were recorded. Selected wells of each cloning were tested by ELISA as above. In the final cloning, positive wells containing single colonies were expanded in RPMI with 11% FBS.

Three antibodies were determined to be reactive for the peptide raised against the amino terminus of CKIαHu (80 G10H11D, 80 H12F12B, and 80 J9E10C), and three antibodies were reactive with the peptide raised against the internal fragment of CKIα3Hu (75 D3G10A, 75 C10H1D, and 75 C2G11F). Clones 75D3G, 75C10H, 75C2G, and 80G10H were isotyped to be IgG1, clone 80H4F IgG3, and 80J9E IgG2a.

B. CKIHu/Thioredoxin Fusion Proteins

Expression plasmids were constructed in order to express the CKIHu isoforms as fusion proteins with thioredoxin. Specifically, the coding sequence for each isoform was amplified by PCR with primers which created a 5´*Xba*I restriction site and a 3´*Bam*HI site. The primer used to create the *Xba*I site for the CKIαHu isoforms is set out in SEQ ID NO: 43 with the *Xba*I site underlined.

5´-T ACA TCT AGA ATT ATG GCG AGT AGC AGC GGC-3´
(SEQ ID NO: 43)

The primer used to create the 3´*Bam*HI site in the CKIα1Hu coding sequence is set out in SEQ ID NO: 44, with *Bam*HI site underlined.

5´-AAT GGA TCC TTA GAA ACC TGT GGG GGT-3´
(SEQ ID NO: 44)

The primer used to create the *Bam*HI site in the CKIα2Hu and CKIα3Hu coding sequences is set out in SEQ ID NO: 45, with the *Bam*HI site underlined.

5´-AAT GGA TCC TTA GAA ACC TTT CAT GTT ACT CTT GGT-3´
(SEQ ID NO: 45)

The *Xba*I and *Bam*HI sites were created in the CKIδHu coding sequences with primers set out in SEQ ID NOS: 46 and 47, respectively.

5´-T ACA TCT AGA ATT ATG GAG CTG AGA GTC GGG-5´
(SEQ ID NO: 46)

5´-GGA TCC TCA TCG GTG CAC GAC AGA CTG-3´
(SEQ ID NO:47)

The primers used to create the *Xba*I and *Bam*HI sites in the coding regions of the CKIγHu isoforms are set out in SEQ ID NO: 48 and 49.

5´T ACA TCT AGA ATT ATG GCA CGA CCT AGT GGT CGA TCG-3´
(SEQ ID NO: 48)

5´-G GGG ATC CTA CTT CAG TAG GGG CTG-3´
(SEQ ID NO: 49)

Digestion of the resulting PCR products with *Xba*I and *Bam*HI allowed the fragments to be directionly cloned in frame at the carboxy terminus of sequences encoding thioredoxin in plasmid pTRXFUS [LeVallie, *et al.*, *Nature/Biotechnology 11*:187-193 (1993)]. The resulting expression constructions contained the *laq* Iq gene, followed by the tacII promoter (from plasmid pMal-c2,

New England Biolabs, Beverly, MA) which drives expression of the *E.coli* thioredoxin gene fused at the amino termini of the CKI catalytic domains.

E.coli XL-1 Blue cells (Stratagene) were transformed with the individual expression plasmids by standard methods and grown at 37°C to mid-log phase. Samples were collected to serve as controls for uninduced cells and the remaining cells were induced for four hours with 0.25 mM IPTG at 37°C. Cells were then lysed and inclusion bodies in the insoluble extract from cleared lysate were used to inject mice.

C. Other CKI Peptides

Monoclonal antibodies were also raised against other CKI peptides coupled to bovine gamma globulin as in section A of this example. Peptides derived from the amino termini of the CKIγHu isoforms are set out in SEQ ID NOS: 50 and 51; peptides derived from the amino termini of bovine CKIβ [Rowles, *et al.*, *supra*] are set out in SEQ ID NOS: 52 and 53; peptides derived from the amino terminus and carboxy terminus of CKIδHu are set out in SEQ ID NOS: 54 and 55, respectively; a peptide derived from the carboxy termini of CKIα2Hu and CKIα3Hu is set out in SEQ ID NO: 56; and a peptide common to all CKIHu isoforms is set out in SEQ ID NO: 57. The common CKI sequence set out in SEQ ID NO: 57 was also injected into rabbits to produce polyclonal antisera.

NH_2-RSGHNTRGTGSS-COOH (SEQ ID NO: 50)

NH_2-RLGHNTRGTGSS-COOH (SEQ ID NO: 51)

NH_2-SSRPKTDVLVG-COOH (SEQ ID NO: 52)

NH_2-KSDNTKSEMKHS-COOH (SEQ ID NO: 53)

NH_2-GTDIAAGE-COOH (SEQ ID NO: 54)

NH_2-ERRDREERLR-COOH (SEQ ID NO: 55)

NH_2-TGKQTDKTKSNMKGY-COOH (SEQ ID NO: 56)

NH_2-DLLGPSLEDLFGY-COOH (SEQ ID NO: 57)

Mice were injected with 50 μg of the peptide/gamma globulin complex on a varying schedule over a period of eight months.

Subsequent to the filing of U.S. Patent Application Serial No. 07/728,783 on July 3, 1991, there have been numerous reports in the scientific literature of the isolation of DNAs encoding *HRR25*-like proteins. For example, Rowles, *et al*, (*Proc. Natl. Acad. Sci. USA*, 88:9548-9592, 1991) reported the purification of a bovine thymus casein kinase I (CKI) enzyme. The sequencing of tryptic fragments reveled nearly 25% of the primary sequence of the enzyme. PCR cloning resulted in development of partial clones coding for the CKI enzyme isolate and a homologue enzyme referred to as CKI-δ. Screening of bovine brain libraries with the partial clones yielded full length cDNAs for the CKI isolate (designated CKIα) and two additional homologues (CKIβ and CKIγ). The deduced sequence for bovine CKIα was noted by Rowles, *et al.*, [*supra*] to be 60% homologous to *HRR25* over its catalytic domain. As noted earlier, a comparison of the bovine CKIα sequence of Rowles, *et al.* to human CKIα1 sequence set out in SEQ. ID. NO. 7 and 8 reveals 100% homology in the catalytic domain.

As another example, Robinson, *et al.* (*Proc. Natl. Acad. Sci. USA*, 89:28-32, 1992) describes the isolation of two *Saccharomyces cerevisiae* genes, YCK1 and YCK2 which encode yeast casein kinase 1 homologues and also describes purification and partial sequencing of a rabbit casein kinase I from a rabbit reticulocyte lysate preparation. *HRR25* was noted to be 50% homologous to YCK1 and YCK2 and 60% homologous to the partial rabbit CKI sequence. As a further example, Wang, *et al.* (*Molecular Biology of the Cell*, 3:275-286, 1992) describes the isolation of a 54 kDa CKI from *S. cerevisiae* and the use of amino acid sequence information therefrom for cloning two yeast cDNAs encoding homologous casein kinase I proteins, CKI1 and CKI2. Comparison of the catalytic domains of the protein encoded by the CKI1 gene produced few alignments revealing greater than 20-25% homology. The closest matches were with *HRR25* (50-56%) and with the three bovine isozymes of Rowles, *et al.* (51-56%). The YCK1 sequence of Robinson, *et al.* corresponds to the CKI2

sequence of Wang, *et al.*; the YCK2 sequence corresponds to CKI1. Brockman, *et al.* (*Proc. Natl. Acad. Sci, USA,* 89:9454-9458, 1992) reported the immunopurification and sequencing of a human erythroid casein kinase I and noted that it was 62% homologous to *HRR25*. As a final example, Graves, *et al.* (*J.Biol.Chem.* 265:6394-6401, 1993) reported the cloning and characterization of a casein kinase I from rat testes. This CKI, designated CKIδ, shared 76% homology at the amino acid level with CKIα isolated from bovine brain and 65% homology with HRR25.

While the foregoing illustrative examples are specifically directed to isolation of "full length" polynucleotides encoding the *HRR25*-like proteins *HRR25*, Hhp1+, Hhp2+, CKIα1Hu, CKIα2Hu, CKIα3Hu, CKIδHu, CKIγ1Hu and CKIγ2Hu, it will be readily understood that the present invention is not limited to those polynucleotides. Rather it embraces all polynucleotides which are comprehended within the class of genes encoding *HRR25*-like proteins characterized protein kinase activity and by homology of 35% or more with the *HRR25* protein through the protein kinase catalytic domain. By way of example, employing information concerning the DNA sequence of *HRR25*, the procedures of Example 7 allowed the isolation partial cDNA clones of expected length from cDNA libraries derived from *Arabidopsis thaliana, Drosophila melanogaster, Xenopus*, chicken, mouse, rat, and human species. These partial cDNAs may, in turn, be employed in the manner of Examples 6 and 7 to isolate full length DNA clones encoding *HRR25*-like proteins from these species. Each of these may be employed in the large scale production of the corresponding proteins by recombinant methods or for the generation of other useful polynucleotides such as antisense RNAs. Recombinant expression products of such *HRR25*-like DNAs may be employed for generation of antibodies and in screens for compounds which modulate the protein kinase and/or recombination/repair functions of these enzymes. Moreover, as suggested in the publication of Rowles, *et al.*, Robinson, *et al.*, and Wang, *et al.*, multiple *HRR25*-like isozymes are expected to exist in a variety of eukaryotic species as both membrane bound and cytoplasmic proteins. It appears reasonable to expect that a number of genes and gene products exist in

human species, all of which are functionally related as well as structurally related to each other and to *HRR25*.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention.

SUMMARY OF SEQUENCES

SEQ ID N0: 1 is the nucleic acid sequence and the deduced amino acid of a genomic fragment encoding a yeast-derived protein kinase, *HRR25* of the present invention.

SEQ ID NO: 2 is the deduced amino acid sequence of a yeast-derived protein kinase *HRR25* of the present invention.

SEQ ID NO: 3 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding Hhp1+ of the present invention.

SEQ ID NO: 4 is the deduced amino acid sequence of Hhp1+ of the present invention.

SEQ ID NO: 5 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding Hhp2+ of the present invention.

SEQ ID NO: 6 is the deduced amino acid sequence of Hhp2+ of the present invention.

SEQ ID NO: 7 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding CK1α1Hu of the present invention.

SEQ ID NO: 8 is the deduced amino acid sequence of CK1α1Hu of the present invention.

SEQ ID NO: 9 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding CK1α2Hu of the present invention.

SEQ ID NO: 10 is the deduced amino acid sequence of CK1α2Hu of the present invention.

SEQ ID NO: 11 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding CK1α3Hu of the present invention.

SEQ ID NO: 12 is the deduced amino acid sequence of CK1α3Hu of the present invention.

SEQ ID NO: 13 is the primer, 4583, representing top strand DNA encoding residues 16-23 of *HRR25*.

SEQ ID NO: 14 is the primer, 4582, representing top strand DNA encoding residues 126-133 of *HRR25*.

SEQ ID NO: 15 is the primer, 4589, representing bottom strand DNA encoding residues 126-133 of *HRR25*.

SEQ ID NO: 16 is the primer, 4590, representing bottom strand DNA encoding residues 194-199 of *HRR25*.

SEQ ID NO: 17 is the primer JH21, representing bovine top strand DNA bases 47-67.

SEQ ID NO: 18 is the primer JH22, representing bovine top strand DNA bases 223-240.

SEQ ID NO: 19 is the primer JH29, representing bovine top strand DNA bases 604-623.

SEQ ID NO: 20 is the primer JH30, representing bovine bottom strand DNA bases 623-604.

SEQ ID NO: 21 is the primer JH31, representing bovine bottom strand DNA bases 835-817.

SEQ ID NO: 22 is the mutated *HRR25* kinase domain primer found on p. 33, Example 3.

SEQ ID NO: 23 is the nucleic acid sequence (and the deduced amino acid sequence) of a genomic fragment encoding NUF1 of the present invention.

SEQ ID NO: 24 is the deduced amino acid sequence of NUF1 of the present invention.

SEQ ID NOS: 25, 26 and 27 are the conserved motifs found on page 18.

SEQ ID NOS: 28 and 29 are redundant oligonucleotides, based on conserved regions of HRR25-like proteins, used to amplify a probe from a human cDNA library.

SEQ ID NO: 30 is the nucleotide sequence of the CKIγ1Hu gene.

SEQ ID NO: 31 is the deduced amino acid sequence of the CKIγ1Hu protein.

SEQ ID NO: 32 is the nucleotide sequence of the CKIγ2Hu gene.

SEQ ID NO: 33 is the deduced amino acid sequence of the CKIγ2Hu protein.

SEQ ID NO: 34 is the nucleic acid sequence for CKIδHu.

SEQ ID NO: 35 is the deduced amino acid sequence for CKIδHu.

SEQ ID NO: 36 is the mutagenic oligonucleotide used to generate an *Nco*I restriction site in expression plasmid pRS305.

SEQ ID NO: 37 is the mutagenic oligonucleotide used to generate an *Nco*I restriction site in CKIγ1.

SEQ ID NO: 38 is the mutagenic oligonucleotide used to create an *Nco*I restriction site in human CKIαa.

SEQ ID NO: 39 is the mutagenic oligonucleotide used to introduce a *Bgl*II restriction site in CKIδ.

SEQ ID NO: 40 is the intervening nucleic acids sequence between the GAL1 promoter and initiating methionine codon in the CKIδ expression plasmid.

SEQ ID NOS: 41 and 42 are amino terminal and internal peptide fragments of CKIα isoforms to generate monoclonal antibodies.

SEQ ID NO: 43 is the primer used to create a *Xba*I restriction site in CKIαHu coding sequences.

SEQ ID NO: 44 is the primer used to create a *Bam*HI restriction site in the CKIα1Hu coding sequence.

SEQ ID NO: 45 is the primer used to create a *Bam*HI restriction site in the CKIα2Hu and CKIα3Hu coding sequences.

SEQ ID NO: 46 is the primer used to create a *Xba*I restriction site in the CKIδHu coding sequence.

SEQ ID NO: 47 is the primer used to create a *Bam*HI restriction site in the CKIδHu coding sequence.

SEQ ID NO: 48 is the primer used to create a *Xba*I restriction site in the CKIγ1Hu and CKI γ2Hu coding sequences.

SEQ ID NO: 49 is the primer used to create a *Bam*HI restriction site in the CKIγ1Hu and CKI γ2Hu coding sequences.

SEQ ID NO: 50 is an amino terminal peptide fragment of CKIγHu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQ ID NO: 51 is an amino terminal peptide fragment of CKIγHu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQ ID NO: 52 is an amino terminal peptide fragment of bovine CKIβ coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQ ID NO: 53 is an amino terminal peptide fragment of bovine CKIβ coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQ ID NO: 54 is an amino terminal peptide fragment of CKIδHu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQ ID NO: 55 is a carboxy terminal peptide fragment of CKIδHu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQ ID NO: 56 is an carboxy terminal peptide fragment of CKIα2Hu and CKIα3Hu coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQ ID NO: 57 is an internal terminal peptide fragment common to all human CKI isoforms coupled to bovine gamma globulin and used to generate monoclonal antibodies in mice.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Salk Institute For Biological Studies

(ii) TITLE OF INVENTION: Protein Kinases

(iii) NUMBER OF SEQUENCES: 57

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/008,001
(B) FILING DATE: 21-JAN-1993

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/728,783
(B) FILING DATE: 03-JUL-1991

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3098 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 879..2360

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GTCGACTCGC CAATCACCAA GTTCTTATCC CACATCCGAC CAGTGTCTGA GTCATGGTTT    60

ACCACCACCA TACCATCGCT GGTCATTTGT AAATCCGTTT CTATTACATC AGCACCTGCT   120

GCATAAGCCT TCTCAAATGC TAGTAGCGTA TTTTCAGGAT ATCTTGCTTT AAAAGCTCTG   180

TGGCCCACAA TTTCAACCAT CCTCGTGTCC TTGTTGTTAT CTTACACTTC TTATTTATCA   240

ATAACACTAG TAACATCAAC AACACCAATT TTATATCTCC CTTAATTGTA TACTAAAAGA   300

TCTAAACCAA TTCGGTATTG TCCTCGATAC GGCATGCGTA TAAAGAGATA TAATTAAAAG   360

AGGTTATAGT CACGTGATGC AGATTACCCG CAACAGTACC ACAAAATGGA TACCATCTAA   420

TTGCTATAAA AGGCTCCTAT ATACGAATAA CTACCACTGG ATCGACGATT ATTTCGTGGC   480

AATCATATAC CACTGTGAAG AGTTACTGCA ACTCTCGCTT TGTTTCAACG CTTCTTCCCG   540

TCTGTGTATT TACTACTAAT AGGCAGCCCA CGTTTGAATT TCTTTTTTTC TGGAGAATTT   600

TTGGTGCAAC GAGGAAAAGG AGACGAAGAA AAAAAGTTGA AACACGACCA CATATATGGA   660

ACGTGGTTGA AATACAAAGA GAAGAAAGGT TCGACACTCG AGGAAAGCAT TTGGTGGTGA   720

AAACACATCT TAGTAGCATC TTTAAACCTC TGTTGGGTAC TTAGAAAAAT ATTTCCAGAC   780

TTCAAGGATA AAAAAAGTCG AAAAGTTACG ACATATTCGA CCAAAAAAAA AAACCAAAAA   840

GAAAAGATAT ATTTATAGAA AGGATACATT AAAAAGAG ATG GAC TTA AGA GTA        893
                                          Met Asp Leu Arg Val
                                            1               5

GGA AGG AAA TTT CGT ATT GGC AGG AAG ATT GGG AGT GGT TCC TTT GGT      941
Gly Arg Lys Phe Arg Ile Gly Arg Lys Ile Gly Ser Gly Ser Phe Gly
                10                  15                  20

GAC ATT TAC CAC GGC ACG AAC TTA ATT AGT GGT GAA GAA GTA GCC ATC      989
Asp Ile Tyr His Gly Thr Asn Leu Ile Ser Gly Glu Glu Val Ala Ile
            25                  30                  35

AAG CTG GAA TCG ATC AGG TCC AGA CAT CCT CAA TTG GAC TAT GAG TCC     1037
Lys Leu Glu Ser Ile Arg Ser Arg His Pro Gln Leu Asp Tyr Glu Ser
        40                  45                  50

CGC GTC TAC AGA TAC TTA AGC GGT GGT GTG GGA ATC CCG TTC ATC AGA     1085
Arg Val Tyr Arg Tyr Leu Ser Gly Gly Val Gly Ile Pro Phe Ile Arg
    55                  60                  65

TGG TTT GGC AGA GAG GGT GAA TAT AAT GCT ATG GTC ATC GAT CTT CTA     1133
Trp Phe Gly Arg Glu Gly Glu Tyr Asn Ala Met Val Ile Asp Leu Leu
70                  75                  80                  85

GGC CCA TCT TTG GAA GAT TTA TTC AAC TAC TGT CAC AGA AGG TTC TCC     1181
Gly Pro Ser Leu Glu Asp Leu Phe Asn Tyr Cys His Arg Arg Phe Ser
                90                  95                  100

TTT AAG ACG GTT ATC ATG CTG GCT TTG CAA ATG TTT TGC CGT ATT CAG     1229
Phe Lys Thr Val Ile Met Leu Ala Leu Gln Met Phe Cys Arg Ile Gln
            105                 110                 115
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TAT ATA CAT GGA AGG TCG TTC ATT CAT AGA GAT ATC AAA CCA GAC AAC      1277
Tyr Ile His Gly Arg Ser Phe Ile His Arg Asp Ile Lys Pro Asp Asn
        120                 125                 130

TTT TTA ATG GGG GTA GGA CGC CGT GGT AGC ACC GTT CAT GTT ATT GAT      1325
Phe Leu Met Gly Val Gly Arg Arg Gly Ser Thr Val His Val Ile Asp
    135                 140                 145

TTC GGT CTA TCA AAG AAA TAC CGA GAT TTC AAC ACA CAT CGT CAT ATT      1373
Phe Gly Leu Ser Lys Lys Tyr Arg Asp Phe Asn Thr His Arg His Ile
150                 155                 160                 165

CCT TAC AGG GAG AAC AAG TCC TTG ACA GGT ACA GCT CGT TAT GCA AGT      1421
Pro Tyr Arg Glu Asn Lys Ser Leu Thr Gly Thr Ala Arg Tyr Ala Ser
                170                 175                 180

GTC AAT ACG CAT CTT GGA ATA GAG CAA AGT AGA AGA GAT GAC TTA GAA      1469
Val Asn Thr His Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Leu Glu
            185                 190                 195

TCA CTA GGT TAT GTC TTG ATC TAT TTT TGT AAG GGT TCT TTG CCA TGG      1517
Ser Leu Gly Tyr Val Leu Ile Tyr Phe Cys Lys Gly Ser Leu Pro Trp
        200                 205                 210

CAG GGT TTG AAA GCA ACC ACC AAG AAA CAA AAG TAT GAT CGT ATC ATG      1565
Gln Gly Leu Lys Ala Thr Thr Lys Lys Gln Lys Tyr Asp Arg Ile Met
    215                 220                 225

GAA AAG AAA TTA AAC GTT AGC GTG GAA ACT CTA TGT TCA GGT TTA CCA      1613
Glu Lys Lys Leu Asn Val Ser Val Glu Thr Leu Cys Ser Gly Leu Pro
230                 235                 240                 245

TTA GAG TTT CAA GAA TAT ATG GCT TAC TGT AAG AAT TTG AAA TTC GAT      1661
Leu Glu Phe Gln Glu Tyr Met Ala Tyr Cys Lys Asn Leu Lys Phe Asp
                250                 255                 260

GAG AAG CCA GAT TAT TTG TTC TTG GCA AGG CTG TTT AAA GAT CTG AGT      1709
Glu Lys Pro Asp Tyr Leu Phe Leu Ala Arg Leu Phe Lys Asp Leu Ser
            265                 270                 275

ATT AAA CTA GAG TAT CAC AAC GAC CAC TTG TTC GAT TGG ACA ATG TTG      1757
Ile Lys Leu Glu Tyr His Asn Asp His Leu Phe Asp Trp Thr Met Leu
        280                 285                 290

CGT TAC ACA AAG GCG ATG GTG GAG AAG CAA AGG GAC CTC CTC ATC GAA      1805
Arg Tyr Thr Lys Ala Met Val Glu Lys Gln Arg Asp Leu Leu Ile Glu
    295                 300                 305

AAA GGT GAT TTG AAC GCA AAT AGC AAT GCA GCA AGT GCA AGT AAC AGC      1853
Lys Gly Asp Leu Asn Ala Asn Ser Asn Ala Ala Ser Ala Ser Asn Ser
310                 315                 320                 325

ACA GAC AAC AAG TCT GAA ACT TTC AAC AAG ATT AAA CTG TTA GCC ATG      1901
Thr Asp Asn Lys Ser Glu Thr Phe Asn Lys Ile Lys Leu Leu Ala Met
                330                 335                 340

AAG AAA TTC CCC ACC CAT TTC CAC TAT TAC AAG AAT GAA GAC AAA CAT      1949
Lys Lys Phe Pro Thr His Phe His Tyr Tyr Lys Asn Glu Asp Lys His
            345                 350                 355

AAT CCT TCA CCA GAA GAG ATC AAA CAA CAA ACT ATC TTG AAT AAT AAT      1997
Asn Pro Ser Pro Glu Glu Ile Lys Gln Gln Thr Ile Leu Asn Asn Asn
        360                 365                 370

GCA GCC TCT TCT TTA CCA GAG GAA TTA TTG AAC GCA CTA GAT AAA GGT      2045
Ala Ala Ser Ser Leu Pro Glu Glu Leu Leu Asn Ala Leu Asp Lys Gly
    375                 380                 385
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ATG GAA AAC TTG AGA CAA CAG CAG CCG CAG CAG CAG GTC CAA AGT TCG      2093
Met Glu Asn Leu Arg Gln Gln Gln Pro Gln Gln Gln Val Gln Ser Ser
390                 395                 400                 405

CAG CCA CAA CCA CAG CCC CAA CAG CTA CAG CAG CAA CCA AAT GGC CAA      2141
Gln Pro Gln Pro Gln Pro Gln Gln Leu Gln Gln Gln Pro Asn Gly Gln
                410                 415                 420

AGA CCA AAT TAT TAT CCT GAA CCG TTA CTA CAG CAG CAA CAA AGA GAT      2189
Arg Pro Asn Tyr Tyr Pro Glu Pro Leu Leu Gln Gln Gln Gln Arg Asp
            425                 430                 435

TCT CAG GAG CAA CAG CAG CAA GTT CCG ATG GCT ACA ACC AGG GCT ACT      2237
Ser Gln Glu Gln Gln Gln Gln Val Pro Met Ala Thr Thr Arg Ala Thr
        440                 445                 450

CAG TAT CCC CCA CAA ATA AAC AGC AAT AAT TTT AAT ACT AAT CAA GCA      2285
Gln Tyr Pro Pro Gln Ile Asn Ser Asn Asn Phe Asn Thr Asn Gln Ala
    455                 460                 465

TCT GTA CCT CCA CAA ATG AGA TCT AAT CCA CAA CAG CCG CCT CAA GAT      2333
Ser Val Pro Pro Gln Met Arg Ser Asn Pro Gln Gln Pro Pro Gln Asp
470                 475                 480                 485

AAA CCA GCT GGC CAG TCA ATT TGG TTG TAAGCAACAT ATATTGCTCA            2380
Lys Pro Ala Gly Gln Ser Ile Trp Leu
                490

AAACGCACAA AAATAAACAT ATGTATATAT AGACATACAC ACACACATAT ATATATATAT    2440

ATTATTATTA TTATTTACAT ATACGTACAC ACAATTCCAT ATCGAGTTAA TATATACAAT    2500

TCTGGCCTTC TTACCTAAAA AGATGATAGC TAAAAGAACC ACTTTTTTTA TGCATTTTTT    2560

TCTTCGGGAA GGAAATTAAG GGGGAGCGGA GCACCTCTTG GCCAATTTGT TTTTTTTTTA    2620

TGTAATAAAG GGCTAACGAT CGAAGATCAA TCACGAATAT TGGACGGTTT TAAAGGAGGG    2680

CCTCTGAGAA GACAGCATCA ATTCGTATTT TCGATAATTA ACTTGCCTTA TAGTGTCTGA    2740

TTAGGAAACA ATCACGAGAC GATAACGACG GAATACCAAG GAAGTTTGTG CAAATATACA    2800

GCCGGCACAA ACAGCAGCTT CACTCAGGTT AACTCACATA CTGTTGAAAA TTGTCGGTAT    2860

GGAATTCGTT GCAGAAAGGG CTCAGCCAGT TGGTCAAACA ATCCAGCAGC AAAATGTTAA    2920

TACTTACGGG CAAGGCGTCC TACAACCGCA TCATGATTTA CAGCAGCGAC AACAACAACA    2980

ACAGCAGCGT CAGCATCAAC AACTGCTGAC GTCTCAGTTG CCCCAGAAAT CTCTCGTATC    3040

CAAAGGCAAA TATACACTAC ATGACTTCCA GATTATGAGA ACGCTTGGTA CTGGATCC      3098
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 494 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Leu Arg Val Gly Arg Lys Phe Arg Ile Gly Arg Lys Ile Gly
 1               5                  10                  15
```

```
Ser Gly Ser Phe Gly Asp Ile Tyr His Gly Thr Asn Leu Ile Ser Gly
                20                  25                  30

Glu Glu Val Ala Ile Lys Leu Glu Ser Ile Arg Ser Arg His Pro Gln
            35                  40                  45

Leu Asp Tyr Glu Ser Arg Val Tyr Arg Tyr Leu Ser Gly Gly Val Gly
        50                  55                  60

Ile Pro Phe Ile Arg Trp Phe Gly Arg Glu Gly Glu Tyr Asn Ala Met
65                  70                  75                  80

Val Ile Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn Tyr Cys
                85                  90                  95

His Arg Arg Phe Ser Phe Lys Thr Val Ile Met Leu Ala Leu Gln Met
            100                 105                 110

Phe Cys Arg Ile Gln Tyr Ile His Gly Arg Ser Phe Ile His Arg Asp
        115                 120                 125

Ile Lys Pro Asp Asn Phe Leu Met Gly Val Gly Arg Arg Gly Ser Thr
    130                 135                 140

Val His Val Ile Asp Phe Gly Leu Ser Lys Lys Tyr Arg Asp Phe Asn
145                 150                 155                 160

Thr His Arg His Ile Pro Tyr Arg Glu Asn Lys Ser Leu Thr Gly Thr
                165                 170                 175

Ala Arg Tyr Ala Ser Val Asn Thr His Leu Gly Ile Glu Gln Ser Arg
            180                 185                 190

Arg Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Ile Tyr Phe Cys Lys
        195                 200                 205

Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala Thr Thr Lys Lys Gln Lys
    210                 215                 220

Tyr Asp Arg Ile Met Glu Lys Lys Leu Asn Val Ser Val Glu Thr Leu
225                 230                 235                 240

Cys Ser Gly Leu Pro Leu Glu Phe Gln Glu Tyr Met Ala Tyr Cys Lys
                245                 250                 255

Asn Leu Lys Phe Asp Glu Lys Pro Asp Tyr Leu Phe Leu Ala Arg Leu
            260                 265                 270

Phe Lys Asp Leu Ser Ile Lys Leu Glu Tyr His Asn Asp His Leu Phe
        275                 280                 285

Asp Trp Thr Met Leu Arg Tyr Thr Lys Ala Met Val Glu Lys Gln Arg
    290                 295                 300

Asp Leu Leu Ile Glu Lys Gly Asp Leu Asn Ala Asn Ser Asn Ala Ala
305                 310                 315                 320

Ser Ala Ser Asn Ser Thr Asp Asn Lys Ser Glu Thr Phe Asn Lys Ile
                325                 330                 335

Lys Leu Leu Ala Met Lys Lys Phe Pro Thr His Phe His Tyr Tyr Lys
            340                 345                 350

Asn Glu Asp Lys His Asn Pro Ser Pro Glu Glu Ile Lys Gln Gln Thr
        355                 360                 365
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Ile Leu Asn Asn Asn Ala Ala Ser Ser Leu Pro Glu Glu Leu Leu Asn
    370                 375                 380

Ala Leu Asp Lys Gly Met Glu Asn Leu Arg Gln Gln Gln Pro Gln Gln
385                 390                 395                 400

Gln Val Gln Ser Ser Gln Pro Gln Pro Gln Pro Gln Gln Leu Gln Gln
                405                 410                 415

Gln Pro Asn Gly Gln Arg Pro Asn Tyr Tyr Pro Glu Pro Leu Leu Gln
            420                 425                 430

Gln Gln Gln Arg Asp Ser Gln Glu Gln Gln Gln Gln Val Pro Met Ala
        435                 440                 445

Thr Thr Arg Ala Thr Gln Tyr Pro Pro Gln Ile Asn Ser Asn Asn Phe
    450                 455                 460

Asn Thr Asn Gln Ala Ser Val Pro Pro Gln Met Arg Ser Asn Pro Gln
465                 470                 475                 480

Gln Pro Pro Gln Asp Lys Pro Ala Gly Gln Ser Ile Trp Leu
                485                 490
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2469 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 113..1207

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AATATTTCAA GCTATACCAA GCATACAATC AACTCCAAGC TTCGAGCGGC CGCCAGTGTG      60

CTCTAAAGGA AAAAGCGAGT GCCTTTAGCC TTAAAAGCGT TATAATATTA TT ATG         115
                                                            Met
                                                             1

GCT TTG GAC CTC CGG ATT GGG AAC AAG TAT CGC ATT GGT CGT AAA ATT       163
Ala Leu Asp Leu Arg Ile Gly Asn Lys Tyr Arg Ile Gly Arg Lys Ile
              5                  10                  15

GGC AGT GGA TCT TTC GGA GAC ATT TAT CTT GGG ACT AAT GTC GTT TCT       211
Gly Ser Gly Ser Phe Gly Asp Ile Tyr Leu Gly Thr Asn Val Val Ser
          20                  25                  30

GGT GAA GAG GTC GCT ATC AAG CTA GAA TCA ACT CGT GCT AAA CAC CCT       259
Gly Glu Glu Val Ala Ile Lys Leu Glu Ser Thr Arg Ala Lys His Pro
      35                  40                  45

CAA TTG GAG TAT GAA TAC AGA GTT TAT CGC ATT TTG TCA GGA GGG GTC       307
Gln Leu Glu Tyr Glu Tyr Arg Val Tyr Arg Ile Leu Ser Gly Gly Val
  50                  55                  60                  65
```

```
GGA ATC CCG TTT GTT CGT TGG TTC GGT GTA GAA TGT GAT TAC AAC GCT          355
Gly Ile Pro Phe Val Arg Trp Phe Gly Val Glu Cys Asp Tyr Asn Ala
                70                  75                  80

ATG GTG ATG GAT TTA TTG GGT CCT TCG TTG GAA GAC TTG TTT AAT TTT          403
Met Val Met Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn Phe
            85                  90                  95

TGC AAT CGA AAG TTT TCT TTG AAA ACA GTT CTT CTC CTT GCG GAC CAG          451
Cys Asn Arg Lys Phe Ser Leu Lys Thr Val Leu Leu Leu Ala Asp Gln
        100                 105                 110

CTC ATT TCT CGA ATT GAA TTC ATT CAT TCA AAA TCT TTT CTT CAT CGT          499
Leu Ile Ser Arg Ile Glu Phe Ile His Ser Lys Ser Phe Leu His Arg
    115                 120                 125

GAT ATT AAG CCT GAT AAC TTT TTA ATG GGA ATA GGT AAA AGA GGA AAT          547
Asp Ile Lys Pro Asp Asn Phe Leu Met Gly Ile Gly Lys Arg Gly Asn
130                 135                 140                 145

CAA GTT AAC ATA ATT GAT TTC GGA TTG GCT AAG AAG TAT CGT GAT CAC          595
Gln Val Asn Ile Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp His
                150                 155                 160

AAA ACT CAC CTG CAC ATT CCT TAT CGC GAG AAC AAG AAT CTT ACA GGT          643
Lys Thr His Leu His Ile Pro Tyr Arg Glu Asn Lys Asn Leu Thr Gly
            165                 170                 175

ACT GCA CGC TAT GCT AGC ATC AAT ACT CAT TTA GGT ATT GAA CAA TCC          691
Thr Ala Arg Tyr Ala Ser Ile Asn Thr His Leu Gly Ile Glu Gln Ser
        180                 185                 190

CGC CGT GAT GAC CTC GAA TCT TTA GGT TAT GTG CTC GTC TAC TTT TGT          739
Arg Arg Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Val Tyr Phe Cys
    195                 200                 205

CGT GGT AGC CTG CCT TGG CAG GGA TTG AAG GCT ACC ACG AAA AAG CAA          787
Arg Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala Thr Thr Lys Lys Gln
210                 215                 220                 225

AAG TAT GAA AAG ATT ATG GAG AAG AAG ATC TCT ACG CCT ACA GAG GTC          835
Lys Tyr Glu Lys Ile Met Glu Lys Lys Ile Ser Thr Pro Thr Glu Val
                230                 235                 240

TTA TGT CGG GGA TTC CCT CAG GAG TTC TCA ATT TAT CTC AAT TAC ACG          883
Leu Cys Arg Gly Phe Pro Gln Glu Phe Ser Ile Tyr Leu Asn Tyr Thr
            245                 250                 255

AGA TCT TTA CGT TTC GAT GAC AAA CCT GAT TAC GCC TAC CTT CGC AAG          931
Arg Ser Leu Arg Phe Asp Asp Lys Pro Asp Tyr Ala Tyr Leu Arg Lys
        260                 265                 270

CTT TTC CGA GAT CTT TTT TGT CGG CAA TCT TAT GAG TTT GAC TAT ATG          979
Leu Phe Arg Asp Leu Phe Cys Arg Gln Ser Tyr Glu Phe Asp Tyr Met
    275                 280                 285

TTT GAT TGG ACC TTG AAG AGA AAG ACT CAA CAA GAC CAA CAA CAT CAG         1027
Phe Asp Trp Thr Leu Lys Arg Lys Thr Gln Gln Asp Gln Gln His Gln
290                 295                 300                 305

CAG CAA TTA CAG CAA CAA CTG TCT GCA ACT CCT CAA GCT ATT AAT CCG         1075
Gln Gln Leu Gln Gln Gln Leu Ser Ala Thr Pro Gln Ala Ile Asn Pro
                310                 315                 320

CCG CCA GAG AGG TCT TCA TTT AGA AAT TAT CAA AAA CAA AAC TTT GAT         1123
Pro Pro Glu Arg Ser Ser Phe Arg Asn Tyr Gln Lys Gln Asn Phe Asp
            325                 330                 335
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GAA AAA GGC GGA GAC ATT AAT ACA ACC GTT CCT GTT ATA AAT GAT CCA      1171
Glu Lys Gly Gly Asp Ile Asn Thr Thr Val Pro Val Ile Asn Asp Pro
        340                 345                 350

TCT GCA ACC GGA GCT CAA TAT ATC AAC AGA CCT AAT TGATTAGCCT          1217
Ser Ala Thr Gly Ala Gln Tyr Ile Asn Arg Pro Asn
    355                 360                 365

TTCATATTAT TATTATATAG CATGGGCACA TTATTTTTAT ATTTTCTTCT CATCTGGAGT   1277

CTTCCAATAC TTGCCTTTTA TCCTCCAGAC GTCCTTTAAT TTTGTTGATA GCGCAGGGCT   1337

TTTTCCTTGG GATGGCGAAA GTTACTTTGC TTATAGTTTA TTGAGGGTTC ATAGCTTATT   1397

TGGCTGAAGA TCTTGTGTTG ACTTAAATTC TATGCTAACC TCATGATCAT ATCCTCATTA   1457

TGGCAAGTTT TGGTGAAAAA TTTTTTAATA TTAGTACATT TGCTAATAAT ACATTTGGTA   1517

TTTGTTTTTA CTACCTGTGA ATCTATTCAT ACATTATCAT ATATGTTTCG AGCCAGGAAC   1577

AGAAAAAAGT GAGAGAATTT TCTGCAGAAA TGATCATAAT TTTATCTTCG CTTAACACGA   1637

ATCCTGGTGA CAGATTATCG TGGTTTAAAG CCTTTTTTTT ACGACGCCAT AAGCAAATTG   1697

GTTACTTTTT TATGTGTGAT GAGCCTTGGG GTTTAATCTA ATTAGAAGGC ATTGCATTCA   1757

TATACTTTTA ATAATATATT ATCAGCTATT TGCTGCTTTT CTTTATAGAT ACCGTCTTTT   1817

CCAAGCTGAA CTCATTTAAT CAGCGTCGTT TAACCTTAGG ATGCTTAAGA TGCGTTTAAA   1877

TTCAATGACT TAATGCTCGA GGGATGAATG GTTTGTTTTA GTTCGTGTTC TGGGTGCATG   1937

ATCTCGTGCT TGACTGTTTT ATTGAAGCGT TCATTTCATG AAGTGTCTTT CGATGTTGTT   1997

CACACTTCTG TTTGCTAAAT ATAATAAATA TTTTGCTTTT CACTTTAGAG CACACTGGCG   2057

GCCGCTCGAA GCTTTGGACT TCTTCGCCAT TGGTCAAGTC TCCAATCAAG GTTGTCGGCT   2117

TGTCTACCTT GCCAGAAATT TACGAAAAGA TGGAAAAGGG ATCCAAATCG TTGGTAGATA   2177

CTTGTTGACA CTTCTAAATA AGCGAATTTC TTATGATTTA TGATTTTTAT TATTAAATAA   2237

GTTATAAAAA AAATAAGGTA TACAAATTTT AAAGTGACTC TTAGGTTTTA AAACGAAAAT   2297

TCTTATTCTT GAGTAACTCT TTCCTGTAGG TCAGGTTGCT TTCTCAGGTA TAGCATGAGG   2357

TCGCTCTTAT TGACCACACC TCTACCGGCA TGCCGAGCAA ATGCCTGCAA ATCGCTCCCC   2417

ATTTCACCCA ATTGTAGATA TGCTAACTCC AGCAATGAGC CGATGAATCT CC            2469
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 365 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Ala Leu Asp Leu Arg Ile Gly Asn Lys Tyr Arg Ile Gly Arg Lys
 1               5                  10                  15

Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr Leu Gly Thr Asn Val Val
            20                  25                  30
```

Ser Gly Glu Glu Val Ala Ile Lys Leu Glu Ser Thr Arg Ala Lys His
35 40 45

Pro Gln Leu Glu Tyr Glu Tyr Arg Val Tyr Arg Ile Leu Ser Gly Gly
50 55 60

Val Gly Ile Pro Phe Val Arg Trp Phe Gly Val Glu Cys Asp Tyr Asn
65 70 75 80

Ala Met Val Met Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn
85 90 95

Phe Cys Asn Arg Lys Phe Ser Leu Lys Thr Val Leu Leu Leu Ala Asp
100 105 110

Gln Leu Ile Ser Arg Ile Glu Phe Ile His Ser Lys Ser Phe Leu His
115 120 125

Arg Asp Ile Lys Pro Asp Asn Phe Leu Met Gly Ile Gly Lys Arg Gly
130 135 140

Asn Gln Val Asn Ile Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp
145 150 155 160

His Lys Thr His Leu His Ile Pro Tyr Arg Glu Asn Lys Asn Leu Thr
165 170 175

Gly Thr Ala Arg Tyr Ala Ser Ile Asn Thr His Leu Gly Ile Glu Gln
180 185 190

Ser Arg Arg Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Val Tyr Phe
195 200 205

Cys Arg Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala Thr Thr Lys Lys
210 215 220

Gln Lys Tyr Glu Lys Ile Met Glu Lys Lys Ile Ser Thr Pro Thr Glu
225 230 235 240

Val Leu Cys Arg Gly Phe Pro Gln Glu Phe Ser Ile Tyr Leu Asn Tyr
245 250 255

Thr Arg Ser Leu Arg Phe Asp Asp Lys Pro Asp Tyr Ala Tyr Leu Arg
260 265 270

Lys Leu Phe Arg Asp Leu Phe Cys Arg Gln Ser Tyr Glu Phe Asp Tyr
275 280 285

Met Phe Asp Trp Thr Leu Lys Arg Lys Thr Gln Gln Asp Gln Gln His
290 295 300

Gln Gln Gln Leu Gln Gln Gln Leu Ser Ala Thr Pro Gln Ala Ile Asn
305 310 315 320

Pro Pro Pro Glu Arg Ser Ser Phe Arg Asn Tyr Gln Lys Gln Asn Phe
325 330 335

Asp Glu Lys Gly Gly Asp Ile Asn Thr Thr Val Pro Val Ile Asn Asp
340 345 350

Pro Ser Ala Thr Gly Ala Gln Tyr Ile Asn Arg Pro Asn
355 360 365

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1989 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 50..1249

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
CCGCCAGTGT GCTCTAAAGG TCATCTCTGT GAATTAGAAT CTTAGCAAA ATG ACG        55
                                                      Met Thr
                                                        1

GTT GTT GAC ATT AAG ATT GGT AAT AAA TAT CGT ATA GGT AGA AAA ATT      103
Val Val Asp Ile Lys Ile Gly Asn Lys Tyr Arg Ile Gly Arg Lys Ile
          5                  10                  15

GGT TCT GGC TCC TTT GGT CAA ATT TAC CTG GGA TTA AAT ACG GTA AAT      151
Gly Ser Gly Ser Phe Gly Gln Ile Tyr Leu Gly Leu Asn Thr Val Asn
     20                  25                  30

GGA GAA CAA GTT GCT GTG AAA TTG GAG CCT TTA AAG GCT CGT CAT CAT      199
Gly Glu Gln Val Ala Val Lys Leu Glu Pro Leu Lys Ala Arg His His
 35                  40                  45                  50

CAG TTA GAA TAT GAG TTT CGT GTG TAT AAT ATT CTT AAA GGA AAT ATT      247
Gln Leu Glu Tyr Glu Phe Arg Val Tyr Asn Ile Leu Lys Gly Asn Ile
                 55                  60                  65

GGC ATA CCC ACA ATT CGC TGG TTC GGT GTA ACC AAT AGT TAT AAT GCT      295
Gly Ile Pro Thr Ile Arg Trp Phe Gly Val Thr Asn Ser Tyr Asn Ala
            70                  75                  80

ATG GTC ATG GAT TTA TTA GGC CCT TCT CTG GAA GAT TTA TTC TGC TAT      343
Met Val Met Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Cys Tyr
        85                  90                  95

TGT GGA AGA AAG TTT ACT CTT AAA ACG GTT CTT TTA CTT GCT GAT CAA      391
Cys Gly Arg Lys Phe Thr Leu Lys Thr Val Leu Leu Leu Ala Asp Gln
    100                 105                 110

CTC ATC AGT CGC ATT GAA TAT GTT CAC TCC AAG TCA TTC TTA CAT CGA      439
Leu Ile Ser Arg Ile Glu Tyr Val His Ser Lys Ser Phe Leu His Arg
115                 120                 125                 130

GAC ATT AAG CCT GAT AAT TTT TTA ATG AAG AAG CAC AGC AAT GTT GTT      487
Asp Ile Lys Pro Asp Asn Phe Leu Met Lys Lys His Ser Asn Val Val
                135                 140                 145

ACG ATG ATT GAC TTC GGA TTG GCG AAA AAA TAC AGG GAT TTT AAA ACT      535
Thr Met Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp Phe Lys Thr
            150                 155                 160
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CAT GTT CAT ATT CCA TAT CGA GAT AAT AAG AAT CTT ACG GGA ACG GCT        583
His Val His Ile Pro Tyr Arg Asp Asn Lys Asn Leu Thr Gly Thr Ala
        165                 170                 175

CGA TAT GCT AGT ATT AAC ACC CAT ATT GGT ATT GAA CAA TCT CGC CGT        631
Arg Tyr Ala Ser Ile Asn Thr His Ile Gly Ile Glu Gln Ser Arg Arg
    180                 185                 190

GAT GAC CTC GAA TCG TTA GGT TAT GTT TTA CTT TAT TTT TGT CGC GGC        679
Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Leu Tyr Phe Cys Arg Gly
195                 200                 205                 210

AGT TTG CCC TGG CAA GGC TTA CAA GCT GAT ACA AAG GAG CAA AAG TAT        727
Ser Leu Pro Trp Gln Gly Leu Gln Ala Asp Thr Lys Glu Gln Lys Tyr
                215                 220                 225

CAA CGG ATA CGT GAT ACC AAG ATT GGC ACT CCT TTG GAA GTC CTT TGC        775
Gln Arg Ile Arg Asp Thr Lys Ile Gly Thr Pro Leu Glu Val Leu Cys
            230                 235                 240

AAA GGT CTT CCC GAA GAG TTT ATC ACT TAC ATG TGT TAC ACT CGT CAG        823
Lys Gly Leu Pro Glu Glu Phe Ile Thr Tyr Met Cys Tyr Thr Arg Gln
        245                 250                 255

CTT TCG TTT ACC GAG AAG CCA AAC TAT GCT TAT TTG AGA AAG CTG TTT        871
Leu Ser Phe Thr Glu Lys Pro Asn Tyr Ala Tyr Leu Arg Lys Leu Phe
    260                 265                 270

CGT GAT TTA CTT ATT CGT AAA GGA TAC CAG TAT GAC TAT GTT TTT GAC        919
Arg Asp Leu Leu Ile Arg Lys Gly Tyr Gln Tyr Asp Tyr Val Phe Asp
275                 280                 285                 290

TGG ATG ATA TTA AAA TAC CAA AAG CGA GCT GCT GCT GCT GCC GCC GCT        967
Trp Met Ile Leu Lys Tyr Gln Lys Arg Ala Ala Ala Ala Ala Ala Ala
                295                 300                 305

TCT GCT ACA GCA CCT CCA CAG GTT ACA TCT CCT ATG GTG TCA CAA ACT       1015
Ser Ala Thr Ala Pro Pro Gln Val Thr Ser Pro Met Val Ser Gln Thr
            310                 315                 320

CAA CCG GTT AAT CCC ATT ACT CCT AAT TAT TCA TCC ATT CCC TTA CCT       1063
Gln Pro Val Asn Pro Ile Thr Pro Asn Tyr Ser Ser Ile Pro Leu Pro
        325                 330                 335

GCT GAG CGG AAT CCA AAG ACT CCA CAA TCT TTC TCC ACT AAT ATT GTT       1111
Ala Glu Arg Asn Pro Lys Thr Pro Gln Ser Phe Ser Thr Asn Ile Val
    340                 345                 350

CAA TGT GCT TCT CCC TCA CCT CTT CCT CTC TCC TTT CGT TCT CCT GTT       1159
Gln Cys Ala Ser Pro Ser Pro Leu Pro Leu Ser Phe Arg Ser Pro Val
355                 360                 365                 370

CCC AAC AAA GAT TAT GAA TAC ATT CCA TCT TCG TTG CAA CCT CAA TAC       1207
Pro Asn Lys Asp Tyr Glu Tyr Ile Pro Ser Ser Leu Gln Pro Gln Tyr
                375                 380                 385

AGT GCT CAA CTG AGG CGT GTT TTA GAT GAA GAA CCA GCT CCT               1249
Ser Ala Gln Leu Arg Arg Val Leu Asp Glu Glu Pro Ala Pro
            390                 395                 400

TGATTTTTTG ACTTTACTTT TCATCAATTC CTCTCTTACA CTACGTCTTT TAGTCTTAAA     1309

TTCCAAACCA TCTGTTGACG TTTTAAAGTT CCACAAATAT CTTTAATAAT TCCTGGCTTT     1369

CTTTTTTGTC TATGGATGGC CGGATTGCTA CACTAATACA CTTTGAGGTT TAGCTATTGT     1429

TTTGAGCTAT TCCATTTTGC CTAGAAGTTG AGTTTTAATG CCTTCTTTTT AAATAGACAT     1489
```

```
ATTGTGTAAA CCTCATACAT GCTTTACTGA AAAGACATAA TTAGAGGACA AAATTTAAAT    1549

CGTGCTGTTT GTTTATATTC AGCTCGTTCC GGTCAAGTTC TTGCCAAAGA ATTGAGTCAG    1609

TCGTGCTATT CATTTCTAAA TTTCTTCTTC CCAGAATTTT ATTTTATTGT TTTCGTTCCC    1669

CATTGGTTCT TACATTCCGT TTTTATTCAA AACTGAAAAG TTTGTACCTC CATTGCTAGA    1729

AGTAATATAC ACAAGGAGCA TGTTTCTTTT TTTACACTAT CATTTGCGTG GCTCTAAACC    1789

AGTCTTTATT GCCTACCTTT GCAATAAAAG ATATAATATC AATTGCATAA GAAATAATTC    1849

ATTAATAAAT GATAAATTTC ATCGATTAAA TAAAAAAAAA AAACTTTAGA GCTTTAGAGC    1909

ACAACTGGCG GCCGCTCGAA GCTTTGGACT TCTTCGCCAT TGGTCAAGTC TCAATCAAGG    1969

TTGTCGGCTT GTCTACCTTC                                               1989
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 400 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Thr Val Val Asp Ile Lys Ile Gly Asn Lys Tyr Arg Ile Gly Arg
 1               5                  10                  15

Lys Ile Gly Ser Gly Ser Phe Gly Gln Ile Tyr Leu Gly Leu Asn Thr
            20                  25                  30

Val Asn Gly Glu Gln Val Ala Val Lys Leu Glu Pro Leu Lys Ala Arg
        35                  40                  45

His His Gln Leu Glu Tyr Glu Phe Arg Val Tyr Asn Ile Leu Lys Gly
    50                  55                  60

Asn Ile Gly Ile Pro Thr Ile Arg Trp Phe Gly Val Thr Asn Ser Tyr
65                  70                  75                  80

Asn Ala Met Val Met Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe
                85                  90                  95

Cys Tyr Cys Gly Arg Lys Phe Thr Leu Lys Thr Val Leu Leu Leu Ala
            100                 105                 110

Asp Gln Leu Ile Ser Arg Ile Glu Tyr Val His Ser Lys Ser Phe Leu
        115                 120                 125

His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met Lys Lys His Ser Asn
    130                 135                 140

Val Val Thr Met Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp Phe
145                 150                 155                 160

Lys Thr His Val His Ile Pro Tyr Arg Asp Asn Lys Asn Leu Thr Gly
                165                 170                 175

Thr Ala Arg Tyr Ala Ser Ile Asn Thr His Ile Gly Ile Glu Gln Ser
            180                 185                 190
```

```
Arg Arg Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Leu Tyr Phe Cys
        195                 200                 205

Arg Gly Ser Leu Pro Trp Gln Gly Leu Gln Ala Asp Thr Lys Glu Gln
    210                 215                 220

Lys Tyr Gln Arg Ile Arg Asp Thr Lys Ile Gly Thr Pro Leu Glu Val
225                 230                 235                 240

Leu Cys Lys Gly Leu Pro Glu Glu Phe Ile Thr Tyr Met Cys Tyr Thr
                245                 250                 255

Arg Gln Leu Ser Phe Thr Glu Lys Pro Asn Tyr Ala Tyr Leu Arg Lys
            260                 265                 270

Leu Phe Arg Asp Leu Leu Ile Arg Lys Gly Tyr Gln Tyr Asp Tyr Val
        275                 280                 285

Phe Asp Trp Met Ile Leu Lys Tyr Gln Lys Arg Ala Ala Ala Ala Ala
    290                 295                 300

Ala Ala Ser Ala Thr Ala Pro Pro Gln Val Thr Ser Pro Met Val Ser
305                 310                 315                 320

Gln Thr Gln Pro Val Asn Pro Ile Thr Pro Asn Tyr Ser Ser Ile Pro
                325                 330                 335

Leu Pro Ala Glu Arg Asn Pro Lys Thr Pro Gln Ser Phe Ser Thr Asn
            340                 345                 350

Ile Val Gln Cys Ala Ser Pro Ser Pro Leu Pro Leu Ser Phe Arg Ser
        355                 360                 365

Pro Val Pro Asn Lys Asp Tyr Glu Tyr Ile Pro Ser Ser Leu Gln Pro
    370                 375                 380

Gln Tyr Ser Ala Gln Leu Arg Arg Val Leu Asp Glu Glu Pro Ala Pro
385                 390                 395                 400
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1210 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 173..1147

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
GGCGGTGATC AGTTCCCCTC TGCTGATTCT GGGCCCGAAC CCGGTAAAGG CCTCCGTGTT      60

CCGTTTCCTG CCGCCCTCCT CCGTAGCCTT GCCTAGTGTA GGAGCCCCGA GGCCTCCGTC      120
```

```
CTCTTCCCAG AGGTGTCGGG GCTTGCCCCA GCCTCCATCT TCGTCTCTCA GG ATG          175
                                                           Met
                                                            1

GCG AGT AGC AGC GGC TCC AAG GCT GAA TTC ATT GTC GGA GGG AAA TAT        223
Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys Tyr
                5                  10                  15

AAA CTG GTA CGG AAG ATC GGG TCT GGC TCC TTC GGG GAC ATC TAT TTG        271
Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr Leu
            20                  25                  30

GCG ATC AAC ATC ACC AAC GGC GAG GAA GTG GCA GTG AAG CTA GAA TCT        319
Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu Ser
        35                  40                  45

CAG AAG GCC AGG CAT CCC CAG TTG CTG TAC GAG AGC AAG CTC TAT AAG        367
Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr Lys
    50                  55                  60                  65

ATT CTT CAA GGT GGG GTT GGC ATC CCC CAC ATA CGG TGG TAT GGT CAG        415
Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly Gln
                70                  75                  80

GAA AAA GAC TAC AAT GTA CTA GTC ATG GAT CTT CTG GGA CCT AGC CTC        463
Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser Leu
            85                  90                  95

GAA GAC CTC TTC AAT TTC TGT TCA AGA AGG TTC ACA ATG AAA ACT GTA        511
Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr Val
        100                 105                 110

CTT ATG TTA GCT GAC CAG ATG ATC AGT AGA ATT GAA TAT GTG CAT ACA        559
Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His Thr
    115                 120                 125

AAG AAT TTT ATA CAC AGA GAC ATT AAA CCA GAT AAC TTC CTA ATG GGT        607
Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met Gly
130                 135                 140                 145

ATT GGG CGT CAC TGT AAT AAG TTA TTC CTT ATT GAT TTT GGT TTG GCC        655
Ile Gly Arg His Cys Asn Lys Leu Phe Leu Ile Asp Phe Gly Leu Ala
                150                 155                 160

AAA AAG TAC AGA GAC AAC AGG ACA AGG CAA CAC ATA CCA TAC AGA GAA        703
Lys Lys Tyr Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg Glu
            165                 170                 175

GAT AAA AAC CTC ACT GGC ACT GCC CGA TAT GCT AGC ATC AAT GCA CAT        751
Asp Lys Asn Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala His
        180                 185                 190

CTT GGT ATT GAG CAG AGT CGC CGA GAT GAC ATG GAA TCA TTA GGA TAT        799
Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly Tyr
    195                 200                 205

GTT TTG ATG TAT TTT AAT AGA ACC AGC CTG CCA TGG CAA GGG CTA AAG        847
Val Leu Met Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu Lys
210                 215                 220                 225

GCT GCA ACA AAG AAA CAA AAA TAT GAA AAG ATT AGT GAA AAG AAG ATG        895
Ala Ala Thr Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys Met
                230                 235                 240

TCC ACG CCT GTT GAA GTT TTA TGT AAG GGG TTT CCT GCA GAA TTT GCG        943
Ser Thr Pro Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe Ala
            245                 250                 255
```

```
ATG TAC TTA AAC TAT TGT CGT GGG CTA CGC TTT GAG GAA GCC CCA GAT      991
Met Tyr Leu Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro Asp
        260                 265                 270

TAC ATG TAT CTG AGG CAG CTA TTC CGC ATT CTT TTC AGG ACC CTG AAC     1039
Tyr Met Tyr Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu Asn
    275                 280                 285

CAT CAA TAT GAC TAC ACA TTT GAT TGG ACA ATG TTA AAG CAG AAA GCA     1087
His Gln Tyr Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys Ala
290                 295                 300                 305

GCA CAG CAG GCA GCC TCT TCC AGT GGG CAG GGT CAG CAG GCC CAA ACC     1135
Ala Gln Gln Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln Thr
                310                 315                 320

CCC ACA GGT TTC TAAGCATGAA TTGAGGAACA GAAGAAGCAG AGCAGATGAT        1187
Pro Thr Gly Phe
            325

CGAGCAGCAT TTGTTTCTCC CAA                                          1210
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 325 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys
  1               5                  10                  15

Tyr Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr
            20                  25                  30

Leu Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu
        35                  40                  45

Ser Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr
    50                  55                  60

Lys Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly
65                  70                  75                  80

Gln Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser
                85                  90                  95

Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr
            100                 105                 110

Val Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His
        115                 120                 125

Thr Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met
    130                 135                 140

Gly Ile Gly Arg His Cys Asn Lys Leu Phe Leu Ile Asp Phe Gly Leu
145                 150                 155                 160

Ala Lys Lys Tyr Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg
                165                 170                 175
```

```
Glu Asp Lys Asn Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala
            180                 185                 190

His Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly
        195                 200                 205

Tyr Val Leu Met Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu
    210                 215                 220

Lys Ala Ala Thr Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys
225                 230                 235                 240

Met Ser Thr Pro Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe
                245                 250                 255

Ala Met Tyr Leu Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro
            260                 265                 270

Asp Tyr Met Tyr Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu
        275                 280                 285

Asn His Gln Tyr Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys
    290                 295                 300

Ala Ala Gln Gln Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln
305                 310                 315                 320

Thr Pro Thr Gly Phe
                325
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2385 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 297..1388

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GAATTCCGAT AGTATTATGT GGAGTTCCAT TTTTATGTAT TTTTTGTATG AAATATTCTA      60

GTATAAGTAA ATATTTTATC AGAAGTATTT ACATATCTTT TTTTTTTTTA GTTTGAGAGC     120

GGCGGTGATC AGGTTCCCCT CTGCTGATTC TGGGCCCCGA ACCCCGGTAA AGGCCTCCGT     180

GTTCCGTTTC CTGCCGCCCT CCTCCGTAGC CTTGCCTAGT GTAGGAGCCC CGAGGCCTCC     240

GTCCTCTTCC CAGAGGTGTC GGGGCTTGGC CCCAGCCTCC ATCTTCGTCT CTCAGG         296

ATG GCG AGT AGC AGC GGC TCC AAG GCT GAA TTC ATT GTC GGA GGG AAA        344
Met Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys
  1               5                  10                  15

TAT AAA CTG GTA CGG AAG ATC GGG TCT GGC TCC TTC GGG GAC ATC TAT       392
Tyr Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr
            20                  25                  30
```

```
TTG GCG ATC AAC ATC ACC AAC GGC GAG GAA GTG GCA GTG AAG CTA GAA          440
Leu Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu
          35                  40                  45

TCT CAG AAG GCC AGG CAT CCC CAG TTG CTG TAC GAG AGC AAG CTC TAT          488
Ser Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr
      50                  55                  60

AAG ATT CTT CAA GGT GGG GTT GGC ATC CCC CAC ATA CGG TGG TAT GGT          536
Lys Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly
 65                  70                  75                  80

CAG GAA AAA GAC TAC AAT GTA CTA GTC ATG GAT CTT CTG GGA CCT AGC          584
Gln Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser
                 85                  90                  95

CTC GAA GAC CTC TTC AAT TTC TGT TCA AGA AGG TTC ACA ATG AAA ACT          632
Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr
            100                 105                 110

GTA CTT ATG TTA GCT GAC CAG ATG ATC AGT AGA ATT GAA TAT GTG CAT          680
Val Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His
        115                 120                 125

ACA AAG AAT TTT ATA CAC AGA GAC ATT AAA CCA GAT AAC TTC CTA ATG          728
Thr Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met
    130                 135                 140

GGT ATT GGG CGT CAC TGT AAT AAG TGT TTA GAA TCT CCA GTG GGG AAG          776
Gly Ile Gly Arg His Cys Asn Lys Cys Leu Glu Ser Pro Val Gly Lys
145                 150                 155                 160

AGG AAA AGA AGC ATG ACT GTT AGT ACT TCT CAG GAC CCA TCT TTC TCA          824
Arg Lys Arg Ser Met Thr Val Ser Thr Ser Gln Asp Pro Ser Phe Ser
                165                 170                 175

GGA TTA AAC CAG TTA TTC CTT ATT GAT TTT GGT TTG GCC AAA AAG TAC          872
Gly Leu Asn Gln Leu Phe Leu Ile Asp Phe Gly Leu Ala Lys Lys Tyr
            180                 185                 190

AGA GAC AAC AGG ACA AGG CAA CAC ATA CCA TAC AGA GAA GAT AAA AAC          920
Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg Glu Asp Lys Asn
        195                 200                 205

CTC ACT GGC ACT GCC CGA TAT GCT AGC ATC AAT GCA CAT CTT GGT ATT          968
Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala His Leu Gly Ile
    210                 215                 220

GAG CAG AGT CGC CGA GAT GAC ATG GAA TCA TTA GGA TAT GTT TTG ATG         1016
Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly Tyr Val Leu Met
225                 230                 235                 240

TAT TTT AAT AGA ACC AGC CTG CCA TGG CAA GGG CTA AAG GCT GCA ACA         1064
Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu Lys Ala Ala Thr
                245                 250                 255

AAG AAA CAA AAA TAT GAA AAG ATT AGT GAA AAG AAG ATG TCC ACG CCT         1112
Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys Met Ser Thr Pro
            260                 265                 270

GTT GAA GTT TTA TGT AAG GGG TTT CCT GCA GAA TTT GCG ATG TAC TTA         1160
Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe Ala Met Tyr Leu
        275                 280                 285

AAC TAT TGT CGT GGG CTA CGC TTT GAG GAA GCC CCA GAT TAC ATG TAT         1208
Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro Asp Tyr Met Tyr
    290                 295                 300
```

```
CTG AGG CAG CTA TTC CGC ATT CTT TTC AGG ACC CTG AAC CAT CAA TAT      1256
Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu Asn His Gln Tyr
305                 310                 315                 320

GAC TAC ACA TTT GAT TGG ACA ATG TTA AAG CAG AAA GCA GCA CAG CAG      1304
Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys Ala Ala Gln Gln
                325                 330                 335

GCA GCC TCT TCC AGT GGG CAG GGT CAG CAG GCC CAA ACC CCC ACA GGC      1352
Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln Thr Pro Thr Gly
            340                 345                 350

AAG CAA ACT GAC AAA ACC AAG AGT AAC ATG AAA GGT TAGTAGCCAA           1398
Lys Gln Thr Asp Lys Thr Lys Ser Asn Met Lys Gly
        355                 360

GAACCAAGTG ACGTTACAGG GAAAAAATTG AATACAAAAT TGGGTAATTC ATTTCTAACA    1458

GTGTTAGATC AAGGAGGTGG TTTTAAAATA CATAAAAATT TGGCTCTGCG TTAAAAAAAA    1518

AAAAGACGTC CTTGGAAAAT TTGACTACTA ACTTTAAACC CAAATGTCCT TGTTCATATA    1578

TATGTATATG TATTTGTATA TACATATATG TGTGTATATT TATATCATTT CTCTTGGGAT    1638

TTTGGGTCAT TTTTTTAACA ACTGCATCTT TTTTACTCAT TCATTAACCC CCTTTCCAAA    1698

AATTTGGTGT TGGGAATATA ATATAATCAA TCAATCCAAA ATCCTAGACC TAACACTTGT    1758

TGATTTCTAA TAATGAATTT GGTTAGCCAT ATTTTGACTT TATTTCAGAC TAACAATGTT    1818

AAGATTTTTT ATTTTGCATG TTAATGCTTT AGCATTTAAA ATGGAAAATT GTGAACATGT    1878

TGTAATTTCA AGAGGTGAGT TTGGCATTAC CCCCAAAGTG TCTATCTTCT CAGTTGCAGA    1938

GCATCTCATT TTCTCTCTTA AATGCTCAAA TAAATGCAAA GCTCAGCACA TCTTTTCTAG    1998

TCACAAAAAT AATTCTTTTA TTTGCAGTTT ACGTATGATC TTAATTTCAA AACGATTTCT    2058

TTGTTTTTGG CTTGATTTTT CACAATGTTG CAAATATCAG GCTCCCAGGG TTTAATGTGG    2118

AATTGAAGTC TGCAGCCAGG CCTTGCAAAT TGAAGGTAAC TGGGGCAAAT GCCATTGAAA    2178

CCGCTAGTCT TATTTCCTTT CTACTTTTCT TTGGCACTCT TACTGCCTGT AAGGAGTAGA    2238

ACTGTTAAGG CACACTGTTG CTATACAGTT AACTCCCATT TTCATGTTTT GTCTTTCTTT    2298

TCCCATTTCT GGGGCTTACC TCCTGATACC TGCTTACTTT CTGGAAGTAG TGGGCAAGTA    2358

AGATTTGGCT CTTGGTTTCT GGAATTC                                        2385
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 364 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys
  1               5                  10                  15

Tyr Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr
            20                  25                  30
```

Leu Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu
35 40 45

Ser Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr
50 55 60

Lys Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly
65 70 75 80

Gln Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser
85 90 95

Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr
100 105 110

Val Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His
115 120 125

Thr Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met
130 135 140

Gly Ile Gly Arg His Cys Asn Lys Cys Leu Glu Ser Pro Val Gly Lys
145 150 155 160

Arg Lys Arg Ser Met Thr Val Ser Thr Ser Gln Asp Pro Ser Phe Ser
165 170 175

Gly Leu Asn Gln Leu Phe Leu Ile Asp Phe Gly Leu Ala Lys Lys Tyr
180 185 190

Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg Glu Asp Lys Asn
195 200 205

Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala His Leu Gly Ile
210 215 220

Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly Tyr Val Leu Met
225 230 235 240

Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu Lys Ala Ala Thr
245 250 255

Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys Met Ser Thr Pro
260 265 270

Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe Ala Met Tyr Leu
275 280 285

Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro Asp Tyr Met Tyr
290 295 300

Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu Asn His Gln Tyr
305 310 315 320

Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys Ala Ala Gln Gln
325 330 335

Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln Thr Pro Thr Gly
340 345 350

Lys Gln Thr Asp Lys Thr Lys Ser Asn Met Lys Gly
355 360

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2914 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 265..1275

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
GAATTCCCGA GAAACAAGTG GCCCAGCCTG GTAACCGCCG AGAAGCCCTT CACAAACTGC      60

GGCCTGGCAA AAAGAAACCT GACTGAGCGG CGGTGATCAG GTTCCCCTCT GCTGATTCTG     120

GGCCCCGAAC CCCGGTAAAG GCCTCCGTGT TCCGTTTCCT GCCGCCCTCC TCCGTAGCCT     180

TGCCTAGTGT AGGAGCCCCG AGGCCTCCGT CCTCTTCCCA GAGGTGTCGG GGCTTGGCCC     240

CAGCCTCCAT CTTCGTCTCT CAGG ATG GCG AGT AGC AGC GGC TCC AAG GCT         291
                           Met Ala Ser Ser Ser Gly Ser Lys Ala
                             1               5

GAA TTC ATT GTC GGA GGG AAA TAT AAA CTG GTA CGG AAG ATC GGG TCT       339
Glu Phe Ile Val Gly Gly Lys Tyr Lys Leu Val Arg Lys Ile Gly Ser
 10                  15                  20                  25

GGC TCC TTC GGG GAC ATC TAT TTG GCG ATC AAC ATC ACC AAC GGC GAG       387
Gly Ser Phe Gly Asp Ile Tyr Leu Ala Ile Asn Ile Thr Asn Gly Glu
                 30                  35                  40

GAA GTG GCA GTG AAG CTA GAA TCT CAG AAG GCC AGG CAT CCC CAG TTG       435
Glu Val Ala Val Lys Leu Glu Ser Gln Lys Ala Arg His Pro Gln Leu
             45                  50                  55

CTG TAC GAG AGC AAG CTC TAT AAG ATT CTT CAA GGT GGG GTT GGC ATC       483
Leu Tyr Glu Ser Lys Leu Tyr Lys Ile Leu Gln Gly Gly Val Gly Ile
         60                  65                  70

CCC CAC ATA CGG TGG TAT GGT CAG GAA AAA GAC TAC AAT GTA CTA GTC       531
Pro His Ile Arg Trp Tyr Gly Gln Glu Lys Asp Tyr Asn Val Leu Val
     75                  80                  85

ATG GAT CTT CTG GGA CCT AGC CTC GAA GAC CTC TTC AAT TTC TGT TCA       579
Met Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn Phe Cys Ser
 90                  95                  100                 105

AGA AGG TTC ACA ATG AAA ACT GTA CTT ATG TTA GCT GAC CAG ATG ATC       627
Arg Arg Phe Thr Met Lys Thr Val Leu Met Leu Ala Asp Gln Met Ile
                110                 115                 120

AGT AGA ATT GAA TAT GTG CAT ACA AAG AAT TTT ATA CAC AGA GAC ATT       675
Ser Arg Ile Glu Tyr Val His Thr Lys Asn Phe Ile His Arg Asp Ile
            125                 130                 135

AAA CCA GAT AAC TTC CTA ATG GGT ATT GGG CGT CAC TGT AAT AAG TTA       723
Lys Pro Asp Asn Phe Leu Met Gly Ile Gly Arg His Cys Asn Lys Leu
        140                 145                 150
```

```
TTC CTT ATT GAT TTT GGT TTG GCC AAA AAG TAC AGA GAC AAC AGG ACA        771
Phe Leu Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp Asn Arg Thr
    155                 160                 165

AGG CAA CAC ATA CCA TAC AGA GAA GAT AAA AAC CTC ACT GGC ACT GCC        819
Arg Gln His Ile Pro Tyr Arg Glu Asp Lys Asn Leu Thr Gly Thr Ala
170                 175                 180                 185

CGA TAT GCT AGC ATC AAT GCA CAT CTT GGT ATT GAG CAG AGT CGC CGA        867
Arg Tyr Ala Ser Ile Asn Ala His Leu Gly Ile Glu Gln Ser Arg Arg
                190                 195                 200

GAT GAC ATG GAA TCA TTA GGA TAT GTT TTG ATG TAT TTT AAT AGA ACC        915
Asp Asp Met Glu Ser Leu Gly Tyr Val Leu Met Tyr Phe Asn Arg Thr
            205                 210                 215

AGC CTG CCA TGG CAA GGG CTA AAG GCT GCA ACA AAG AAA CAA AAA TAT        963
Ser Leu Pro Trp Gln Gly Leu Lys Ala Ala Thr Lys Lys Gln Lys Tyr
        220                 225                 230

GAA AAG ATT AGT GAA AAG AAG ATG TCC ACG CCT GTT GAA GTT TTA TGT       1011
Glu Lys Ile Ser Glu Lys Lys Met Ser Thr Pro Val Glu Val Leu Cys
    235                 240                 245

AAG GGG TTT CCT GCA GAA TTT GCG ATG TAC TTA AAC TAT TGT CGT GGG       1059
Lys Gly Phe Pro Ala Glu Phe Ala Met Tyr Leu Asn Tyr Cys Arg Gly
250                 255                 260                 265

CTA CGC TTT GAG GAA GCC CCA GAT TAC ATG TAT CTG AGG CAG CTA TTC       1107
Leu Arg Phe Glu Glu Ala Pro Asp Tyr Met Tyr Leu Arg Gln Leu Phe
                270                 275                 280

CGC ATT CTT TTC AGG ACC CTG AAC CAT CAA TAT GAC TAC ACA TTT GAT       1155
Arg Ile Leu Phe Arg Thr Leu Asn His Gln Tyr Asp Tyr Thr Phe Asp
            285                 290                 295

TGG ACA ATG TTA AAG CAG AAA GCA GCA CAG CAG GCA GCC TCT TCC AGT       1203
Trp Thr Met Leu Lys Gln Lys Ala Ala Gln Gln Ala Ala Ser Ser Ser
        300                 305                 310

GGG CAG GGT CAG CAG GCC CAA ACC CCC ACA GGC AAG CAA ACT GAC AAA       1251
Gly Gln Gly Gln Gln Ala Gln Thr Pro Thr Gly Lys Gln Thr Asp Lys
    315                 320                 325

ACC AAG AGT AAC ATG AAA GGT TTC TAAGCATGAA TTGAGGAACA GAAGAAGCAG      1305
Thr Lys Ser Asn Met Lys Gly Phe
330                 335

AGCAGATGAT CGGAGCAGCA TTTGTTTCTC CCCAAATCTA GAAATTTTAG TTCATATGTA     1365

CACTAGCCAG TGGTTGTGGA CAACCATTTA CTTGGTGTAA AGAACTTAAT TTCAGTATAA     1425

ACTGACTCTG GGCAGCATTG GTGATGCTGT ATCCTGAGTT GTAGCCTCTG TAATTGTGAA     1485

TATTAACTGA GATAGTGAAA CATGGTGTCC GGTTTTCTAT TGCATTTTTT CAAGTGGAAA     1545

AGTTAACTAA ATGGTTGACA CACAAAAATT GGTGGAGAAA TTGTGCATAT GCCAATTTTT     1605

TGTTAAAACC TTTTGTTTTG AACTATACTG CTTTGAGATC TCATTTCAGA AGAACGGCAT     1665

GAACAGTCTT CAGCCACAGT TGTGATGGTT GTTAAATGCT CACAATTGTG CATTCTTAGG     1725

GTTTTTCCAT CCCTGGGGTT TGCAAGTTGT TCACTTAAAA CATTCTTAAA ATGGTTGGCT     1785

TCTTGTCTGC AAGCCAGCTG ATATGGTAGC AACCAAAGAT TCCAGTGTTT GAGCATATGA     1845

AAGACTCTGC CTGCTTAATT GTGCTAGAAA TAACAGCATC TAAAGTGAAG ACTTAAGAAA     1905
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AACTTAGTGA CTACTAGATT ATCCTTAGGA CTCTGCATTA ACTCTATAAT GTTCTTGGTA 1965

TTAAAAAAAA AGCATATTTG TCACAGAAAT TTAGTTAACA TCTTACAACT GAACATGTAT 2025

GTATGTTGCT TAGATAAATG TAATCACTGT AAACATCTAT ATGATCTGGG ATTTTGTTTT 2085

TATTTTGAAA TGGGAGCTTT TTTGTTTACA AGTTCATTAA AAACTAAAAA CTGTTTCTGT 2145

AAGGAAATGA GATTTTTTTT AAACAACAAA AAATGCCTTG CTGACTCACT ATTAAATAAA 2205

AATCTCCCCA ATTTTTTGAT AGACTACTTC AAGCCATTTG TTACATGGTA TTCCTTTGCA 2265

AGTCAATTTA GGTTTCGTGT TATAACTTTT CCTCTTTTTT TAAGAAAAAT GAAAAAAGTA 2325

ATTCTTTTGT CTGAAGGGGA AAGGCATTCT TTCATTTTTT TCTTTTTTTT TTTTTTTTTT 2385

TTATGACTTG CAGGCACAAT ATCTAGTACT GCAACTGCCA GAACTTGGTA TTGTAGCTGC 2445

TGCCCGCTGA CTAGCAGCTG GACTGATTTT GAATAAAAAT GAAAGCAGTA CTGGGATTAC 2505

AGGTGAGCCA CAGTGCCTGG CCCTTTTTTG TTTTTATTGT CTGTCTCCCC ACTAGAAGGT 2565

ACGCTCTACA AGGGCAGGGA TTTGTGCATC TTATTCATAG TGTTTCCCAC GTGGCAGATG 2625

CTCACTAAAG ATTTCAAAGG AGAAACTGTG ATGGACTCGT TCTGTAGATG AGAGAACAGA 2685

GGCACAGAGA CCTGTCCATG GTCCCCTGGC AGAAGGAGGT GGGGTCTGGA TTCCACCCCA 2745

GGGCTGCGTG GCTGCAGGAC CTCAGTGCTT GACTCCACAC TGCTGAGGGC TGTGAGTCCC 2805

TGGCCAGCCC AGACACAGTC CTGCAGCCCA GGCTGAGCAT TCTCAGACCT TCATGGAGAT 2865

GCCCACTCTC CTGTGAGCCT CCTGCTTCCT TTGCCCAGGG CCGGAATTC 2914

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 337 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Lys
 1               5                  10                  15

Tyr Lys Leu Val Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile Tyr
            20                  25                  30

Leu Ala Ile Asn Ile Thr Asn Gly Glu Glu Val Ala Val Lys Leu Glu
        35                  40                  45

Ser Gln Lys Ala Arg His Pro Gln Leu Leu Tyr Glu Ser Lys Leu Tyr
    50                  55                  60

Lys Ile Leu Gln Gly Gly Val Gly Ile Pro His Ile Arg Trp Tyr Gly
65                  70                  75                  80

Gln Glu Lys Asp Tyr Asn Val Leu Val Met Asp Leu Leu Gly Pro Ser
                85                  90                  95

Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Arg Phe Thr Met Lys Thr
            100                 105                 110
```

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Val Leu Met Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Val His
        115                 120                 125

Thr Lys Asn Phe Ile His Arg Asp Ile Lys Pro Asp Asn Phe Leu Met
    130                 135                 140

Gly Ile Gly Arg His Cys Asn Lys Leu Phe Leu Ile Asp Phe Gly Leu
145                 150                 155                 160

Ala Lys Lys Tyr Arg Asp Asn Arg Thr Arg Gln His Ile Pro Tyr Arg
                165                 170                 175

Glu Asp Lys Asn Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn Ala
            180                 185                 190

His Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Met Glu Ser Leu Gly
        195                 200                 205

Tyr Val Leu Met Tyr Phe Asn Arg Thr Ser Leu Pro Trp Gln Gly Leu
    210                 215                 220

Lys Ala Ala Thr Lys Lys Gln Lys Tyr Glu Lys Ile Ser Glu Lys Lys
225                 230                 235                 240

Met Ser Thr Pro Val Glu Val Leu Cys Lys Gly Phe Pro Ala Glu Phe
                245                 250                 255

Ala Met Tyr Leu Asn Tyr Cys Arg Gly Leu Arg Phe Glu Glu Ala Pro
            260                 265                 270

Asp Tyr Met Tyr Leu Arg Gln Leu Phe Arg Ile Leu Phe Arg Thr Leu
        275                 280                 285

Asn His Gln Tyr Asp Tyr Thr Phe Asp Trp Thr Met Leu Lys Gln Lys
    290                 295                 300

Ala Ala Gln Gln Ala Ala Ser Ser Ser Gly Gln Gly Gln Gln Ala Gln
305                 310                 315                 320

Thr Pro Thr Gly Lys Gln Thr Asp Lys Thr Lys Ser Asn Met Lys Gly
                325                 330                 335

Phe
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..23
(D) OTHER INFORMATION: /note= "Bases designated N at positi ns 3, 6, 9, 12 and 18 are Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGNWSNGGNW SNTTYGGNGA YAT 23

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..23
(D) OTHER INFORMATION: /note= "Bases designated N at positions 6, 12 and 18 are Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CAYGMNGAYA TNAARCCNGA YAA 23

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24
(D) OTHER INFORMATION: /note= "Bases designated N at positions 7, 13 and 19 are Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

RTTRTCNGGY TTNATRTCNC KRTG 24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /note= "Bases designated N at positions 1, 4, 7 and 13 are Inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

NCCNARNSWY TCNARRTC 18

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATATAAACTG GTACGGAAGA 20

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..17

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ACATACGGTG GTATGGT 17

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATGACATGGA ATCATTAGG 19

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCTAATGATT CCATGTCAT 19

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCAGGTACAT GTAATCCG 18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..39

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCTGATCGAT TCCAGCCTGA TCGCTACTTC TTCACCACT 39

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3627 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1633..3204

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GATCAGATGA TATAGCTTTT TGTGTGCCGT ACCTTTCCGC GATTCTGCCC GTATATCTTG 60

GTCCCTGAGC TATTTTCTGA GATTCTTTTT GTTGCTTTGC CAAATCATTG GCGTCATTCA 120

TGGTCATACC AAATCCCAAT TTGGCAAACT TGGGTGTTAA AGTATCTTGC TGTTCTTTTC 180

TAGTTGTGTC GAAGCTGTTT GAAGTGTCAT TTAAAAAATC ATTGAATTCA TCAGGCTGGG 240

TATTAATATC ATCTATACTG TTATTATTGT TGCCTTTACT GTTATTCATA AATTGGGAAT 300

CGTAATCATT TGTCTAATTT TGGTGCTAGA AGACGAATTA GTGAACTCGT CCTCCTTTTC 360

TTGTTGAGCC TCTTTTTTAA ATTGATCAAA CAAGTCTTCT GCCTGTGATT TGTCGACTTT 420

CTTTGCGGTT AGTCTAGTGG GCTTTCTTGA CGAAGACAAA ATTGAATGTT TCTTTTTATC 480

TTGCGAGTTT AATACCGGTT TCTTTCTGCA TGCCGTTAAG ATGGAACTCT CGTTTTAGTG 540

ACAGTGGTCT TGGGTGTGCT GCCTGTGGTG TTGTTTTTTG GGGCGAGAGA GCCTGTATTT 600

ACATTGAGTT TAGAACTGGA ATTGGAGCTT GGTTTTTGCC AATTAGAGAA AAAATCGTCA 660

ACACTATTTT CTTTGGAAGT CGACCTGGAA GCGTCTGAAT CGGTGTCCAA CGGTGAGTCC 720

GAAGAATCTT GACCGTTCAA GACTAATTCT GATGGGTATA ACTCCATATC CTTTTGAACC 780

TTCTTGTCGA GATGTATCTT ATATTTCTTA GCAACAGGGC TCGTATATTT TGTTTTCGCG 840

TCAACATTTG CTGTATTTAG TAGCTGTTTC CCATTGTTCT TTAAGAAAAA ATCACGAGCC 900

TTATGGTTCC CACCCAACTT AAACCTTCTT AAATTGTTAA TTGTCCATTT ATCTAATGTA 960

GAAGACTTTA CAAAGGTGAT ATGAACACCC ATGTTTCTAT GCACAGCAGA GCATTGAATA 1020

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CACAGCATCA CACCAAAAGG TACCGAAGTC CAGTAGGATT CTTGTTACCA CAATCAAAAC    1080

AAACTCGATT TTCCATGTTG CTACCTAGCT TCTGAAAAAC TTGTTGAGTA GTCTGTTCCG    1140

TGGCAAATGT TTCTCCTTCA TCGTTACTCA TTGTCGCTAT GTGTATACTA AATTGCTCAA    1200

GAAGACCGGA TCAACAAGTA CTTAACAAAT ACCCTTTCTT TGCTATCGCC TTGATCTCCT    1260

TTTATAAAAT GCCAGCTAAA TCGTGTTTAC GAAGAATAGT TGTTTTCTTT TTTTTTTTT     1320

TTTTTCGAAA CTTTACCGTG TCGTCGAAAA TGACCAAACG ATGTTACTTT TCCTTTTGTG    1380

TCATAGATAA TACCAATATT GAAAGTAAAA TTTTAAACAT TCTATAGGTG AATTGAAAAG    1440

GGCAGCTTAG AGAGTAACAG GGGAACAGCA TTCGTAACAT CTAGGTACTG GTATTATTTG    1500

CTGTTTTTTA AAAAAGAAGG AAATCCGTTT TGCAAGAATT GTCTGCTATT TAAGGGTATA    1560

CGTGCTACGG TCCACTAATC AAAAGTGGTA TCTCATTCTG AAGAAAAAGT GTAAAAAGGA    1620

CGATAAGGAA AG ATG TCC CAA CGA TCT TCA CAA CAC ATT GTA GGT ATT          1668
              Met Ser Gln Arg Ser Ser Gln His Ile Val Gly Ile
                1               5                  10

CAT TAT GCT GTA GGA CCT AAG ATT GGC GAA GGG TCT TTC GGA GTA ATA       1716
His Tyr Ala Val Gly Pro Lys Ile Gly Glu Gly Ser Phe Gly Val Ile
          15                  20                  25

TTT GAG GGA GAG AAC ATT CTT CAT TCT TGT CAA GCG CAG ACC GGT AGC      1764
Phe Glu Gly Glu Asn Ile Leu His Ser Cys Gln Ala Gln Thr Gly Ser
      30                  35                  40

AAG AGG GAC TCT AGT ATA ATA ATG GCG AAC GAG CCA GTC GCA ATT AAA      1812
Lys Arg Asp Ser Ser Ile Ile Met Ala Asn Glu Pro Val Ala Ile Lys
 45                  50                  55                  60

TTC GAA CCG CGA CAT TCG GAC GCA CCC CAG TTG CGT GAC GAA TTT AGA      1860
Phe Glu Pro Arg His Ser Asp Ala Pro Gln Leu Arg Asp Glu Phe Arg
                 65                  70                  75

GCC TAT AGG ATA TTG AAT GGC TGC GTT GGA ATT CCC CAT GCT TAT TAT      1908
Ala Tyr Arg Ile Leu Asn Gly Cys Val Gly Ile Pro His Ala Tyr Tyr
            80                  85                  90

TTT GGT CAA GAA GGT ATG CAC AAC ATC TTG ATT ATC GAT TTA CTA GGG      1956
Phe Gly Gln Glu Gly Met His Asn Ile Leu Ile Ile Asp Leu Leu Gly
          95                  100                 105

CCA TCA TTG GAA GAT CTC TTT GAG TGG TGT GGT AGA AAA TTT TCA GTG      2004
Pro Ser Leu Glu Asp Leu Phe Glu Trp Cys Gly Arg Lys Phe Ser Val
     110                 115                 120

AAA ACA ACC TGT ATG GTT GCC AAG CAA ATG ATT GAT AGA GTT AGA GCA      2052
Lys Thr Thr Cys Met Val Ala Lys Gln Met Ile Asp Arg Val Arg Ala
125                 130                 135                 140

ATT CAT GAT CAC GAC TTA ATC TAT CGC GAT ATT AAA CCC GAT AAC TTT      2100
Ile His Asp His Asp Leu Ile Tyr Arg Asp Ile Lys Pro Asp Asn Phe
                145                 150                 155

TTA ATT TCT CAA TAT CAA AGA ATT TCA CCT GAA GGA AAA GTC ATT AAA      2148
Leu Ile Ser Gln Tyr Gln Arg Ile Ser Pro Glu Gly Lys Val Ile Lys
            160                 165                 170

TCA TGT GCC TCC TCT TCT AAT AAT GAT CCC AAT TTA ATA TAC ATG GTT      2196
Ser Cys Ala Ser Ser Ser Asn Asn Asp Pro Asn Leu Ile Tyr Met Val
        175                 180                 185
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GAC TTT GGT ATG GCA AAA CAA TAT AGA GAT CCA AGA ACG AAA CAA CAT      2244
Asp Phe Gly Met Ala Lys Gln Tyr Arg Asp Pro Arg Thr Lys Gln His
    190                 195                 200

ATA CCA TAC CGT GAA CGA AAA TCA TTG AGC GGT ACC GCC AGA TAT ATG      2292
Ile Pro Tyr Arg Glu Arg Lys Ser Leu Ser Gly Thr Ala Arg Tyr Met
205                 210                 215                 220

TCT ATT AAT ACT CAT TTT GGA AGA GAA CAG TCA CGT AGG GAT GAT TTA      2340
Ser Ile Asn Thr His Phe Gly Arg Glu Gln Ser Arg Arg Asp Asp Leu
                225                 230                 235

GAA TCG CTA GGT CAC GTT TTT TTT TAT TTC TTG AGG GGA TCC TTG CCA      2388
Glu Ser Leu Gly His Val Phe Phe Tyr Phe Leu Arg Gly Ser Leu Pro
            240                 245                 250

TGG CAA GGT TTG AAA GCA CCA AAC AAC AAA CTG AAG TAT GAA AAG ATT      2436
Trp Gln Gly Leu Lys Ala Pro Asn Asn Lys Leu Lys Tyr Glu Lys Ile
        255                 260                 265

GGT ATG ACT AAA CAG AAA TTG AAT CCT GAT GAT CTT TTA TTG AAT AAT      2484
Gly Met Thr Lys Gln Lys Leu Asn Pro Asp Asp Leu Leu Leu Asn Asn
    270                 275                 280

GCT ATT CCT TAT CAG TTT GCC ACA TAT TTA AAA TAT GCA CGT TCC TTG      2532
Ala Ile Pro Tyr Gln Phe Ala Thr Tyr Leu Lys Tyr Ala Arg Ser Leu
285                 290                 295                 300

AAG TTC GAC GAA GAT CCG GAT TAT GAC TAT TTA ATC TCG TTA ATG GAT      2580
Lys Phe Asp Glu Asp Pro Asp Tyr Asp Tyr Leu Ile Ser Leu Met Asp
                305                 310                 315

GAC GCT TTG AGA TTA AAC GAC TTA AAG GAT GAT GGA CAC TAT GAC TGG      2628
Asp Ala Leu Arg Leu Asn Asp Leu Lys Asp Asp Gly His Tyr Asp Trp
            320                 325                 330

ATG GAT TTG AAT GGT GGT AAA GGC TGG AAT ATC AAG ATT AAT AGA AGA      2676
Met Asp Leu Asn Gly Gly Lys Gly Trp Asn Ile Lys Ile Asn Arg Arg
        335                 340                 345

GCT AAC TTG CAT GGT TAC GGA AAT CCA AAT CCA AGA GTC AAT GGC AAT      2724
Ala Asn Leu His Gly Tyr Gly Asn Pro Asn Pro Arg Val Asn Gly Asn
    350                 355                 360

ACT GCA AGA AAC AAT GTG AAT ACG AAT TCA AAG ACA CGA AAT ACA ACG      2772
Thr Ala Arg Asn Asn Val Asn Thr Asn Ser Lys Thr Arg Asn Thr Thr
365                 370                 375                 380

CCA GTT GCG ACA CCT AAG CAA CAA GCT CAA AAC AGT TAT AAC AAG GAC      2820
Pro Val Ala Thr Pro Lys Gln Gln Ala Gln Asn Ser Tyr Asn Lys Asp
                385                 390                 395

AAT TCG AAA TCC AGA ATT TCT TCG AAC CCG CAG AGC TTT ACT AAA CAA      2868
Asn Ser Lys Ser Arg Ile Ser Ser Asn Pro Gln Ser Phe Thr Lys Gln
            400                 405                 410

CAA CAC GTC TTG AAA AAA ATC GAA CCC AAT AGT AAA TAT ATT CCT GAA      2916
Gln His Val Leu Lys Lys Ile Glu Pro Asn Ser Lys Tyr Ile Pro Glu
        415                 420                 425

ACA CAT TCA AAT CTT CAA CGG CCA ATT AAA AGT CAA AGT CAA ACG TAC      2964
Thr His Ser Asn Leu Gln Arg Pro Ile Lys Ser Gln Ser Gln Thr Tyr
    430                 435                 440

GAC TCC ATC AGT CAT ACA CAA AAT TCA CCA TTT GTA CCA TAT TCA AGT      3012
Asp Ser Ile Ser His Thr Gln Asn Ser Pro Phe Val Pro Tyr Ser Ser
445                 450                 455                 460
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TCT AAA GCT AAC CCT AAA AGA AGT AAT AAT GAG CAC AAC TTA CCA AAC      3060
Ser Lys Ala Asn Pro Lys Arg Ser Asn Asn Glu His Asn Leu Pro Asn
                465                 470                 475

CAC TAC ACA AAC CTT GCA AAT AAG AAT ATC AAT TAT CAA AGT CAA CGA      3108
His Tyr Thr Asn Leu Ala Asn Lys Asn Ile Asn Tyr Gln Ser Gln Arg
            480                 485                 490

AAT TAC GAA CAA GAA AAT GAT GCT TAT TCT GAT GAC GAG AAT GAT ACA      3156
Asn Tyr Glu Gln Glu Asn Asp Ala Tyr Ser Asp Asp Glu Asn Asp Thr
        495                 500                 505

TTT TGT TCT AAA ATA TAC AAA TAT TGT TGT TGC TGT TTT TGT TGC TGT      3204
Phe Cys Ser Lys Ile Tyr Lys Tyr Cys Cys Cys Cys Phe Cys Cys Cys
    510                 515                 520

TGATAAAGCG ATTTTTATAC TTTTCTCTTT TTCCTTTTTT TTTTTGATTG GCTGTTTCCT    3264

TATGCCGCTC TTTCCCAATT TATGACTTTC CAATAATGTA TTATTTTGTT TCTCTTTCTC    3324

TCTGTTACCC TTTATTTTAT CATCTACAAT AATTGAATTC CGGAGAGGGT AAAGAAACAG    3384

GAAAAAGAAG AAAATGAGAC ATAGTCAGCA TCGTAATCGT TTTCCTTCTG TATATTCCTT    3444

TATCAAAAGA CTACACGCAC ATATATATTA ATCCCGGTAT GTTTTTGGTG TGCTAAATCT    3504

ATCTTCAAGC ACTATTATAG CATTTTTTTA AGAATATCCA AAATAATATG TAATTTATGA    3564

TTAATCAAGG TTCAAGAATT GGAGAAACCG TGAGCGACTT CTTTGATACT TGGATGTAAG    3624

CTT                                                                  3627
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 524 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Met Ser Gln Arg Ser Ser Gln His Ile Val Gly Ile His Tyr Ala Val
 1               5                  10                  15

Gly Pro Lys Ile Gly Glu Gly Ser Phe Gly Val Ile Phe Glu Gly Glu
            20                  25                  30

Asn Ile Leu His Ser Cys Gln Ala Gln Thr Gly Ser Lys Arg Asp Ser
        35                  40                  45

Ser Ile Ile Met Ala Asn Glu Pro Val Ala Ile Lys Phe Glu Pro Arg
    50                  55                  60

His Ser Asp Ala Pro Gln Leu Arg Asp Glu Phe Arg Ala Tyr Arg Ile
65                  70                  75                  80

Leu Asn Gly Cys Val Gly Ile Pro His Ala Tyr Tyr Phe Gly Gln Glu
                85                  90                  95

Gly Met His Asn Ile Leu Ile Ile Asp Leu Leu Gly Pro Ser Leu Glu
            100                 105                 110

Asp Leu Phe Glu Trp Cys Gly Arg Lys Phe Ser Val Lys Thr Thr Cys
        115                 120                 125
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Met Val Ala Lys Gln Met Ile Asp Arg Val Arg Ala Ile His Asp His
    130                 135                 140

Asp Leu Ile Tyr Arg Asp Ile Lys Pro Asp Asn Phe Leu Ile Ser Gln
145                 150                 155                 160

Tyr Gln Arg Ile Ser Pro Glu Gly Lys Val Ile Lys Ser Cys Ala Ser
                165                 170                 175

Ser Ser Asn Asn Asp Pro Asn Leu Ile Tyr Met Val Asp Phe Gly Met
            180                 185                 190

Ala Lys Gln Tyr Arg Asp Pro Arg Thr Lys Gln His Ile Pro Tyr Arg
        195                 200                 205

Glu Arg Lys Ser Leu Ser Gly Thr Ala Arg Tyr Met Ser Ile Asn Thr
    210                 215                 220

His Phe Gly Arg Glu Gln Ser Arg Arg Asp Asp Leu Glu Ser Leu Gly
225                 230                 235                 240

His Val Phe Phe Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly Leu
                245                 250                 255

Lys Ala Pro Asn Asn Lys Leu Lys Tyr Glu Lys Ile Gly Met Thr Lys
            260                 265                 270

Gln Lys Leu Asn Pro Asp Asp Leu Leu Leu Asn Asn Ala Ile Pro Tyr
        275                 280                 285

Gln Phe Ala Thr Tyr Leu Lys Tyr Ala Arg Ser Leu Lys Phe Asp Glu
    290                 295                 300

Asp Pro Asp Tyr Asp Tyr Leu Ile Ser Leu Met Asp Asp Ala Leu Arg
305                 310                 315                 320

Leu Asn Asp Leu Lys Asp Asp Gly His Tyr Asp Trp Met Asp Leu Asn
                325                 330                 335

Gly Gly Lys Gly Trp Asn Ile Lys Ile Asn Arg Arg Ala Asn Leu His
            340                 345                 350

Gly Tyr Gly Asn Pro Asn Pro Arg Val Asn Gly Asn Thr Ala Arg Asn
        355                 360                 365

Asn Val Asn Thr Asn Ser Lys Thr Arg Asn Thr Thr Pro Val Ala Thr
    370                 375                 380

Pro Lys Gln Gln Ala Gln Asn Ser Tyr Asn Lys Asp Asn Ser Lys Ser
385                 390                 395                 400

Arg Ile Ser Ser Asn Pro Gln Ser Phe Thr Lys Gln Gln His Val Leu
                405                 410                 415

Lys Lys Ile Glu Pro Asn Ser Lys Tyr Ile Pro Glu Thr His Ser Asn
            420                 425                 430

Leu Gln Arg Pro Ile Lys Ser Gln Ser Gln Thr Tyr Asp Ser Ile Ser
        435                 440                 445

His Thr Gln Asn Ser Pro Phe Val Pro Tyr Ser Ser Ser Lys Ala Asn
    450                 455                 460

Pro Lys Arg Ser Asn Asn Glu His Asn Leu Pro Asn His Tyr Thr Asn
465                 470                 475                 480
```

```
Leu Ala Asn Lys Asn Ile Asn Tyr Gln Ser Gln Arg Asn Tyr Glu Gln
                485                 490                 495

Glu Asn Asp Ala Tyr Ser Asp Asp Glu Asn Asp Thr Phe Cys Ser Lys
            500                 505                 510

Ile Tyr Lys Tyr Cys Cys Cys Cys Phe Cys Cys Cys
        515                 520
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Gly Pro Ser Leu Glu Asp
1               5
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..9

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Arg Asp Ile Lys Pro Asp Asn Phe Leu
1               5
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: Protein Kinase

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..6

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

His Ile Pro Tyr Arg Glu
1 5

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 6
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 15
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 21
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GARYTNMGNY TNGGNAAYYT N 21

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: singl
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 9
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 12
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 15
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 18
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 21
(C) OTHER INFORMATION: /note= "The nucleotide at this position is inosine."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTYTTRTTNC CNGGNCKNCC NAT 23

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2405 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 67..1197

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AAAGTGGAGT ACCGCAAACT TGATATGGAA AATAAAAAGA AAGACAAGGA CAAATCAGAT 60

GATAGA ATG GCA CGA CCT AGT GGT CGA TCG GGA CAC AAC ACT CGA GGA 108
Met Ala Arg Pro Ser Gly Arg Ser Gly His Asn Thr Arg Gly
1 5 10

```
ACT GGG TCT TCA TCG TCT GGA GTT TTA ATG GTT GGA CCT AAC TTT AGA        156
Thr Gly Ser Ser Ser Ser Gly Val Leu Met Val Gly Pro Asn Phe Arg
 15                  20                  25                  30

GTT GGA AAA AAA ATT GGA TGT GGC AAT TTT GGA GAA TTA CGA TTA GGG        204
Val Gly Lys Lys Ile Gly Cys Gly Asn Phe Gly Glu Leu Arg Leu Gly
                 35                  40                  45

AAA AAT TTA TAC ACA AAT GAA TAT GTG GCA ATT AAG TTG GAG CCC ATG        252
Lys Asn Leu Tyr Thr Asn Glu Tyr Val Ala Ile Lys Leu Glu Pro Met
             50                  55                  60

AAA TCA AGA GCA CCA CAG CTA CAT TTG GAA TAC AGA TTC TAT AAG CAG        300
Lys Ser Arg Ala Pro Gln Leu His Leu Glu Tyr Arg Phe Tyr Lys Gln
         65                  70                  75

TTA GGA TCT GGA GAT GGT ATA CCT CAA GTT TAC TAT TTC GGC CCC TGT        348
Leu Gly Ser Gly Asp Gly Ile Pro Gln Val Tyr Tyr Phe Gly Pro Cys
     80                  85                  90

GGT AAA TAC AAT GCT ATG GTG CTG GAA CTG CTG GGA CCT AGT TTG GAA        396
Gly Lys Tyr Asn Ala Met Val Leu Glu Leu Leu Gly Pro Ser Leu Glu
 95                  100                 105                 110

GAC TTG TTT GAC TTG TGT GAC AGA ACA TTT TCT CTT AAA ACA GTT CTC        444
Asp Leu Phe Asp Leu Cys Asp Arg Thr Phe Ser Leu Lys Thr Val Leu
                115                 120                 125

ATG ATA GCT ATA CAA CTG ATT TCT CGC ATG GAA TAT GTC CAT TCA AAG        492
Met Ile Ala Ile Gln Leu Ile Ser Arg Met Glu Tyr Val His Ser Lys
            130                 135                 140

AAC TTG ATA TAC AGA GAT GTA AAA CCT GAG AAC TTC TTA ATA GGA CGA        540
Asn Leu Ile Tyr Arg Asp Val Lys Pro Glu Asn Phe Leu Ile Gly Arg
        145                 150                 155

CCA GGA AAC AAA ACC CAG CAA GTT ATT CAC ATT ATA GAT TTT GGT TTG        588
Pro Gly Asn Lys Thr Gln Gln Val Ile His Ile Ile Asp Phe Gly Leu
    160                 165                 170

GCA AAG GAA TAT ATT GAT CCG GAG ACA AAG AAA CAC ATA CCA TAC AGA        636
Ala Lys Glu Tyr Ile Asp Pro Glu Thr Lys Lys His Ile Pro Tyr Arg
175                 180                 185                 190

GAA CAC AAG AGC CTT ACA GGA ACA GCT AGA TAT ATG AGC ATA AAC ACA        684
Glu His Lys Ser Leu Thr Gly Thr Ala Arg Tyr Met Ser Ile Asn Thr
                195                 200                 205

CAT TTA GGA AAA GAA CAA AGT AGA AGA GAC GAT TTA GAA GCT TTA GGT        732
His Leu Gly Lys Glu Gln Ser Arg Arg Asp Asp Leu Glu Ala Leu Gly
            210                 215                 220

CAT ATG TTC ATG TAT TTT CTG AGA GGC AGT CTT CCT TGG CAA GGC TTA        780
His Met Phe Met Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly Leu
        225                 230                 235

AAG GCT GAC ACA TTA AAG GAG AGG TAT CAG AAA ATT GGA GAT ACA AAA        828
Lys Ala Asp Thr Leu Lys Glu Arg Tyr Gln Lys Ile Gly Asp Thr Lys
    240                 245                 250

CGG GCT ACA CCA ATA GAA GTG TTA TGT GAA AAT TTT CCA GAA GAA ATG        876
Arg Ala Thr Pro Ile Glu Val Leu Cys Glu Asn Phe Pro Glu Glu Met
255                 260                 265                 270

GCA ACA TAT CTT CGT TAT GTA AGA AGG CTA GAT TTT TTT GAA AAA CCA        924
Ala Thr Tyr Leu Arg Tyr Val Arg Arg Leu Asp Phe Phe Glu Lys Pro
                275                 280                 285
```

```
GAC TAT GAC TAC TTA AGA AAG CTT TTT ACT GAC TTG TTT GAT CGA AAA      972
Asp Tyr Asp Tyr Leu Arg Lys Leu Phe Thr Asp Leu Phe Asp Arg Lys
            290                 295                 300

GGA TAT ATG TTT GAT TAT GAA TAT GAC TGG ATT GGT AAA CAG TTG CCT     1020
Gly Tyr Met Phe Asp Tyr Glu Tyr Asp Trp Ile Gly Lys Gln Leu Pro
        305                 310                 315

ACT CCA GTG GGT GCA GTT CAG CAA GAT CCT GCT CTG TCA TCA AAC AGA     1068
Thr Pro Val Gly Ala Val Gln Gln Asp Pro Ala Leu Ser Ser Asn Arg
    320                 325                 330

GAA GCA CAT CAA CAC AGA GAT AAG ATG CAA CAA TCC AAA AAC CAG GTT     1116
Glu Ala His Gln His Arg Asp Lys Met Gln Gln Ser Lys Asn Gln Val
335                 340                 345                 350

GTA AGT TCT ACA AAT GGA GAG TTA AAC ACA GAT GAC CCC ACC GCA GAC     1164
Val Ser Ser Thr Asn Gly Glu Leu Asn Thr Asp Asp Pro Thr Ala Asp
                355                 360                 365

GTT CAA ATG CAC CCA TCA CAG CCC CTA CTG AAG TAGAAGTGAT GGATGAAACC   1217
Val Gln Met His Pro Ser Gln Pro Leu Leu Lys
            370                 375

AACTGCCAGA AAGTGTTGAA CATGTGGTGC TGCTGTTTTT TCAAACGAAG GAAAAGGAAA   1277

ACCATACAGC GCCACAAATG ACTCTGGACA CAGACAGATC CTGGGGAGTT ACTTACATGT   1337

TCATCTGCTG TCTTGTGATT AAAATCATCT CTGTAGTGAC CACGTATATT TTCAAGGACT   1397

CACTCTTAGA AACAAAAATG TCATACTTTC ATACTTCATT TTGTGGTTGT CTTACATTCT   1457

TTTTCTTTTT TTTTTTCTCT AATTTAACCT TTATGGAAGC TTTAAAGTTT TGTCAAAAAC   1517

ATGAGTGCTT TTGCCCCATC AGTGAATGGA ATGGACCAAT GAGGTGGTAT CAATGAATAT   1577

AGTTCCATAG AACATTTCCA GAAGTTCTTC TGTTGTAGAA AGCAGTACAG TATCTTAAGT   1637

GTCAACCAGT TATATACCTA ATCTGGTTTT TTATAACTTC TGTAAGAGCA TAATCAAACA   1697

GGAATTTTCT TTTCTCAGTG GATAATACAA CAGAGAAAAC AGAGTTGCCC AAATATTTAA   1757

AAGAAGTTAT TCCTTGAGAA GTTCATATTT TGTGACATCT GCATTGATTT CAGTATTACT   1817

GATGGTACTG TTATTCATAA GTCATATTAA CATTCTCTCC GTGAAATCAT GGTACAGTCG   1877

CTGCCCAGAG GTACTGAGGA AAAAGCAATA TGGGTTCGGC AGATGGTGGT GGTAAAATGA   1937

ATCTTAAGGA GTGTGGTAAA TATGCGTCCG CTTTTGTTGC ATCACTATGT GAAGTACTGT   1997

GTTGCAGAAG TGGCAAAAGC GCTTATTTTT AAAAATGCAA AATATTTGTA CAATGTAACT   2057

TTATGCTTCC AAATAATAAT GTATGTTAGA CAGCAAGAAA TGAATACTTT AAAAAGTGAT   2117

GTATGTTGGA GTTATAAAGA AATACACTAA GGAGAGGTAG TAAATGTGAA CCTTGTTGCA   2177

GTGTATAAGG TGGAAGCCTA AAGAAATCTC ACCGAAACTT ACTGCTGAAT GATTACATTC   2237

TCCCTTAAGC AGAAAACTTT GGATGTGCCA TGCAATGGTG TCTGTGTAAT TATTTTGCTC   2297

TTTGATTAAA AAAAAGACCC CCAGCAATAA AAAGTGGGTC ACTCTAAAAA AAAAAAAAAA   2357

AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA ACGACAGCAA CGGAATTC               2405
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 377 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
Met Ala Arg Pro Ser Gly Arg Ser Gly His Asn Thr Arg Gly Thr Gly
 1               5                  10                  15

Ser Ser Ser Ser Gly Val Leu Met Val Gly Pro Asn Phe Arg Val Gly
            20                  25                  30

Lys Lys Ile Gly Cys Gly Asn Phe Gly Glu Leu Arg Leu Gly Lys Asn
        35                  40                  45

Leu Tyr Thr Asn Glu Tyr Val Ala Ile Lys Leu Glu Pro Met Lys Ser
    50                  55                  60

Arg Ala Pro Gln Leu His Leu Glu Tyr Arg Phe Tyr Lys Gln Leu Gly
65                  70                  75                  80

Ser Gly Asp Gly Ile Pro Gln Val Tyr Tyr Phe Gly Pro Cys Gly Lys
                85                  90                  95

Tyr Asn Ala Met Val Leu Glu Leu Leu Gly Pro Ser Leu Glu Asp Leu
            100                 105                 110

Phe Asp Leu Cys Asp Arg Thr Phe Ser Leu Lys Thr Val Leu Met Ile
        115                 120                 125

Ala Ile Gln Leu Ile Ser Arg Met Glu Tyr Val His Ser Lys Asn Leu
    130                 135                 140

Ile Tyr Arg Asp Val Lys Pro Glu Asn Phe Leu Ile Gly Arg Pro Gly
145                 150                 155                 160

Asn Lys Thr Gln Gln Val Ile His Ile Ile Asp Phe Gly Leu Ala Lys
                165                 170                 175

Glu Tyr Ile Asp Pro Glu Thr Lys Lys His Ile Pro Tyr Arg Glu His
            180                 185                 190

Lys Ser Leu Thr Gly Thr Ala Arg Tyr Met Ser Ile Asn Thr His Leu
        195                 200                 205

Gly Lys Glu Gln Ser Arg Arg Asp Asp Leu Glu Ala Leu Gly His Met
    210                 215                 220

Phe Met Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala
225                 230                 235                 240

Asp Thr Leu Lys Glu Arg Tyr Gln Lys Ile Gly Asp Thr Lys Arg Ala
                245                 250                 255

Thr Pro Ile Glu Val Leu Cys Glu Asn Phe Pro Glu Glu Met Ala Thr
            260                 265                 270

Tyr Leu Arg Tyr Val Arg Arg Leu Asp Phe Phe Glu Lys Pro Asp Tyr
        275                 280                 285

Asp Tyr Leu Arg Lys Leu Phe Thr Asp Leu Phe Asp Arg Lys Gly Tyr
    290                 295                 300
```

```
Met Ph  Asp Tyr Glu Tyr Asp Trp Ile Gly Lys Gln Leu Pro Thr Pro
305                 310                 315                 320

Val Gly Ala Val Gln Gln Asp Pro Ala Leu Ser Ser Asn Arg Glu Ala
                325                 330                 335

His Gln His Arg Asp Lys Met Gln Gln Ser Lys Asn Gln Val Val Ser
            340                 345                 350

Ser Thr Asn Gly Glu Leu Asn Thr Asp Asp Pro Thr Ala Asp Val Gln
        355                 360                 365

Met His Pro Ser Gln Pro Leu Leu Lys
    370                 375
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1233 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1041

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
AGA GTT GGA AAA AAA ATT GGA TGT GGC AAT TTT GGA GAA TTA CGA TTA        48
Arg Val Gly Lys Lys Ile Gly Cys Gly Asn Phe Gly Glu Leu Arg Leu
  1               5                  10                  15

GGG AAA AAT TTA TAC ACA AAT GAA TAT GTG GCA ATT AAG TTG GAG CCC        96
Gly Lys Asn Leu Tyr Thr Asn Glu Tyr Val Ala Ile Lys Leu Glu Pro
            20                  25                  30

ATG AAA TCA AGA GCA CCA CAG CTA CAT TTG GAA TAC AGA TTC TAT AAG       144
Met Lys Ser Arg Ala Pro Gln Leu His Leu Glu Tyr Arg Phe Tyr Lys
        35                  40                  45

CAG TTA GGA TCT GGA GAT GGT ATA CCT CAA GTT TAC TAT TTC GGC CCT       192
Gln Leu Gly Ser Gly Asp Gly Ile Pro Gln Val Tyr Tyr Phe Gly Pro
    50                  55                  60

TGT GGT AAA TAC AAT GCT ATG GTG CTG GAA CTG CTG GGA CCT AGT TTG       240
Cys Gly Lys Tyr Asn Ala Met Val Leu Glu Leu Leu Gly Pro Ser Leu
65                  70                  75                  80

GAA GAC TTG TTT GAC TTG TGT GAC AGA ACA TTT TCT CTT AAA ACA GTT       288
Glu Asp Leu Phe Asp Leu Cys Asp Arg Thr Phe Ser Leu Lys Thr Val
                85                  90                  95

CTC ATG ATA GCT ATA CAA CTG ATT TCT CGC ATG GAA TAT GTC CAT TCA       336
Leu Met Ile Ala Ile Gln Leu Ile Ser Arg Met Glu Tyr Val His Ser
            100                 105                 110

AAG AAC TTG ATA TAC AGA GAT GTA AAA CCT GAG AAC TTC TTA ATA GGA       384
Lys Asn Leu Ile Tyr Arg Asp Val Lys Pro Glu Asn Phe Leu Ile Gly
        115                 120                 125
```

```
CGA CCA GGA AAC AAA ACC CAG CAA GTT ATT CAC ATT ATA GAT TTT GGT        432
Arg Pro Gly Asn Lys Thr Gln Gln Val Ile His Ile Ile Asp Phe Gly
    130                 135                 140

TTG GCA AAG GAA TAT ATT GAT CCG GAG ACA AAG AAA CAC ATA CCA TAC        480
Leu Ala Lys Glu Tyr Ile Asp Pro Glu Thr Lys Lys His Ile Pro Tyr
145                 150                 155                 160

AGA GAA CAC AAG AGC CTT ACA GGA ACA GCT AGA TAT ATG AGC ATA AAC        528
Arg Glu His Lys Ser Leu Thr Gly Thr Ala Arg Tyr Met Ser Ile Asn
                165                 170                 175

ACA CAT TTA GGA AAA GAA CAA AGT AGA AGA GAC GAT TTA GAA GCT TTA        576
Thr His Leu Gly Lys Glu Gln Ser Arg Arg Asp Asp Leu Glu Ala Leu
            180                 185                 190

GGT CAT ATG TTC ATG TAT TTT CTG AGA GGC AGT CTT CCT TGG CAA GGC        624
Gly His Met Phe Met Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly
        195                 200                 205

TTA AAG GTT GAC ACA TTA AAG GAG AGG TAT CAG AAA ATT GGA GAT ACA        672
Leu Lys Val Asp Thr Leu Lys Glu Arg Tyr Gln Lys Ile Gly Asp Thr
    210                 215                 220

AAA CGG GCT ACA CCA ATA GAA GTG TTA TGT GAA AAT TTT CCA GAA ATG        720
Lys Arg Ala Thr Pro Ile Glu Val Leu Cys Glu Asn Phe Pro Glu Met
225                 230                 235                 240

GCA ACA TAT CTT CGT TAT GTA AGA AGG CTA GAT TTT TTT GAA AAA CCA        768
Ala Thr Tyr Leu Arg Tyr Val Arg Arg Leu Asp Phe Phe Glu Lys Pro
                245                 250                 255

GAC TAT GAC TAC TTA AGA AAG CTT TTT ACT GAC TTG TTT GAT CGA AAA        816
Asp Tyr Asp Tyr Leu Arg Lys Leu Phe Thr Asp Leu Phe Asp Arg Lys
            260                 265                 270

GGA TAT ATG TTT GAT TAT GAA TAT GAC TGG ATT GGT AAA CAG TTG CCT        864
Gly Tyr Met Phe Asp Tyr Glu Tyr Asp Trp Ile Gly Lys Gln Leu Pro
        275                 280                 285

ACT CCA GTG GGT GCA GTT CAG CAA GAT CCT GCT CTG TCA TCA AAC AGA        912
Thr Pro Val Gly Ala Val Gln Gln Asp Pro Ala Leu Ser Ser Asn Arg
    290                 295                 300

GAA GCA CAT CAA CAC AGA GAT AAG ATG CAA CAA TCC AAA AAC CAG GTT        960
Glu Ala His Gln His Arg Asp Lys Met Gln Gln Ser Lys Asn Gln Val
305                 310                 315                 320

GTA AGT TCT ACA AAT GGA GAG TTA AAC ACA GAT GAC CCC ACC GCA GAC       1008
Val Ser Ser Thr Asn Gly Glu Leu Asn Thr Asp Asp Pro Thr Ala Asp
                325                 330                 335

GTT CAA ATG CAC CCA TCA CAG CCC CTA CTG AAG TAGAAGTGAT GGATGAAACC      1061
Val Gln Met His Pro Ser Gln Pro Leu Leu Lys
            340                 345

AACTGCCAGA AAGTGTTGAA CATGTGGTGC TGCTGTTTTT TCAAACGAAG GAAAAGGAAA      1121

ACCATACAGC GCCACAAATG ACTCTGGACA CAGACAGATC CTGGGGAGTT ACTTACATGT      1181

TCATCTGCTG TCTTGTGATT AAATCATCTC TGTAGTGACC ACGTATATTT TC              1233
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 347 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Arg Val Gly Lys Lys Ile Gly Cys Gly Asn Phe Gly Glu Leu Arg Leu
  1               5                  10                  15

Gly Lys Asn Leu Tyr Thr Asn Glu Tyr Val Ala Ile Lys Leu Glu Pro
            20                  25                  30

Met Lys Ser Arg Ala Pro Gln Leu His Leu Glu Tyr Arg Phe Tyr Lys
        35                  40                  45

Gln Leu Gly Ser Gly Asp Gly Ile Pro Gln Val Tyr Tyr Phe Gly Pro
    50                  55                  60

Cys Gly Lys Tyr Asn Ala Met Val Leu Glu Leu Leu Gly Pro Ser Leu
65                  70                  75                  80

Glu Asp Leu Phe Asp Leu Cys Asp Arg Thr Phe Ser Leu Lys Thr Val
                85                  90                  95

Leu Met Ile Ala Ile Gln Leu Ile Ser Arg Met Glu Tyr Val His Ser
            100                 105                 110

Lys Asn Leu Ile Tyr Arg Asp Val Lys Pro Glu Asn Phe Leu Ile Gly
        115                 120                 125

Arg Pro Gly Asn Lys Thr Gln Gln Val Ile His Ile Ile Asp Phe Gly
    130                 135                 140

Leu Ala Lys Glu Tyr Ile Asp Pro Glu Thr Lys Lys His Ile Pro Tyr
145                 150                 155                 160

Arg Glu His Lys Ser Leu Thr Gly Thr Ala Arg Tyr Met Ser Ile Asn
                165                 170                 175

Thr His Leu Gly Lys Glu Gln Ser Arg Arg Asp Asp Leu Glu Ala Leu
            180                 185                 190

Gly His Met Phe Met Tyr Phe Leu Arg Gly Ser Leu Pro Trp Gln Gly
        195                 200                 205

Leu Lys Val Asp Thr Leu Lys Glu Arg Tyr Gln Lys Ile Gly Asp Thr
    210                 215                 220

Lys Arg Ala Thr Pro Ile Glu Val Leu Cys Glu Asn Phe Pro Glu Met
225                 230                 235                 240

Ala Thr Tyr Leu Arg Tyr Val Arg Arg Leu Asp Phe Phe Glu Lys Pro
                245                 250                 255

Asp Tyr Asp Tyr Leu Arg Lys Leu Phe Thr Asp Leu Phe Asp Arg Lys
            260                 265                 270

Gly Tyr Met Phe Asp Tyr Glu Tyr Asp Trp Ile Gly Lys Gln Leu Pro
        275                 280                 285

Thr Pro Val Gly Ala Val Gln Gln Asp Pro Ala Leu Ser Ser Asn Arg
    290                 295                 300
```

```
Glu Ala His Gln His Arg Asp Lys Met Gln Gln Ser Lys Asn Gln Val
305                 310                 315                 320

Val Ser Ser Thr Asn Gly Glu Leu Asn Thr Asp Asp Pro Thr Ala Asp
                325                 330                 335

Val Gln Met His Pro Ser Gln Pro Leu Leu Lys
            340                 345
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3505 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 154..1398

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
GAATTCCGAC AGGAAAGCGA TGGTGAAAGC GGGGCCGTGA GGGGGGCGGA GCCGGGAGCC      60

GGACCCGCAG TAGCGGCAGC AGCGGCGCCG CCTCCCGGAG TTCAGACCCA GGAAGCGGCC     120

GGGAGGGCAG GAGCGAATCG GGCCGCCGCC GCC ATG GAG CTG AGA GTC GGG AAC      174
                                      Met Glu Leu Arg Val Gly Asn
                                        1               5

AGG TAC CGG CTG GGC CGG AAG ATC GGC AGC GGC TCC TTC GGA GAC ATC      222
Arg Tyr Arg Leu Gly Arg Lys Ile Gly Ser Gly Ser Phe Gly Asp Ile
        10                  15                  20

TAT CTC GGT ACG GAC ATT GCT GCA GGA GAA GAG GTT GCC ATC AAG CTT      270
Tyr Leu Gly Thr Asp Ile Ala Ala Gly Glu Glu Val Ala Ile Lys Leu
    25                  30                  35

GAA TGT GTC AAA ACC AAA CAC CCT CAG CTC CAC ATT GAG AGC AAA ATC      318
Glu Cys Val Lys Thr Lys His Pro Gln Leu His Ile Glu Ser Lys Ile
40                  45                  50                  55

TAC AAG ATG ATG CAG GGA GGA GTG GGC ATC CCC ACC ATC AGA TGG TGC      366
Tyr Lys Met Met Gln Gly Gly Val Gly Ile Pro Thr Ile Arg Trp Cys
                60                  65                  70

GGG GCA GAG GGG GAC TAC AAC GTC ATG GTG ATG GAG CTG CTG GGG CCA      414
Gly Ala Glu Gly Asp Tyr Asn Val Met Val Met Glu Leu Leu Gly Pro
            75                  80                  85

AGC CTG GAG GAC CTC TTC AAC TTC TGC TCC AGG AAA TTC AGC CTC AAA      462
Ser Leu Glu Asp Leu Phe Asn Phe Cys Ser Arg Lys Phe Ser Leu Lys
        90                  95                  100

ACC GTC CTG CTG CTT GCT GAC CAA ATG ATC AGT CGC ATC GAA TAC ATT      510
Thr Val Leu Leu Leu Ala Asp Gln Met Ile Ser Arg Ile Glu Tyr Ile
    105                 110                 115

CAT TCA AAG AAC TTC ATC CAC CGG GAT GTG AAG CCA GAC AAC TTC CTC      558
His Ser Lys Asn Phe Ile His Arg Asp Val Lys Pro Asp Asn Phe Leu
120                 125                 130                 135
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ATG GGC CTG GGG AAG AAG GGC AAC CTG GTG TAC ATC ATC GAC TTC GGG          606
Met Gly Leu Gly Lys Lys Gly Asn Leu Val Tyr Ile Ile Asp Phe Gly
                140                 145                 150

CTG GCC AAG AAG TAC CGG GAT GCA CGC ACC CAC CAG CAC ATC CCC TAT          654
Leu Ala Lys Lys Tyr Arg Asp Ala Arg Thr His Gln His Ile Pro Tyr
            155                 160                 165

CGT GAG AAC AAG AAC CTC ACG GGG ACG GCG CGG TAC GCC TCC ATC AAC          702
Arg Glu Asn Lys Asn Leu Thr Gly Thr Ala Arg Tyr Ala Ser Ile Asn
        170                 175                 180

ACG CAC CTT GGA ATT GAA CAA TCC CGA AGA GAT GAC TTG GAG TCT CTG          750
Thr His Leu Gly Ile Glu Gln Ser Arg Arg Asp Asp Leu Glu Ser Leu
    185                 190                 195

GGC TAC GTG CTA ATG TAC TTC AAC CTG GGC TCT CTC CCC TGG CAG GGG          798
Gly Tyr Val Leu Met Tyr Phe Asn Leu Gly Ser Leu Pro Trp Gln Gly
200                 205                 210                 215

CTG AAG GCT GCC ACC AAG AGA CAG AAA TAC GAA AGG ATT AGC GAG AAG          846
Leu Lys Ala Ala Thr Lys Arg Gln Lys Tyr Glu Arg Ile Ser Glu Lys
                220                 225                 230

AAA ATG TCC ACC CCC ATC GAA GTG TTG TGT AAA GGC TAC CCT TCC GAA          894
Lys Met Ser Thr Pro Ile Glu Val Leu Cys Lys Gly Tyr Pro Ser Glu
            235                 240                 245

TTT GCC ACA TAC CTG AAT TTC TGC CGT TCC TTG CGT TTT GAC GAC AAG          942
Phe Ala Thr Tyr Leu Asn Phe Cys Arg Ser Leu Arg Phe Asp Asp Lys
        250                 255                 260

CCT GAC TAC TCG TAC CTG CGG CAG CTT TTC CGG AAT CTG TTC CAT CGC          990
Pro Asp Tyr Ser Tyr Leu Arg Gln Leu Phe Arg Asn Leu Phe His Arg
    265                 270                 275

CAG GGC TTC TCC TAT GAC TAC GTG TTC GAC TGG AAC ATG CTC AAA TTT         1038
Gln Gly Phe Ser Tyr Asp Tyr Val Phe Asp Trp Asn Met Leu Lys Phe
280                 285                 290                 295

GGT GCC AGC CGG GCC GCC GAT GAC GCC GAG CGG GAG CGC AGG GAC CGA         1086
Gly Ala Ser Arg Ala Ala Asp Asp Ala Glu Arg Glu Arg Arg Asp Arg
                300                 305                 310

GAG GAG CGG CTG AGA CAC TCG CGG AAC CCG GCT ACC CGC GGC CTC CCT         1134
Glu Glu Arg Leu Arg His Ser Arg Asn Pro Ala Thr Arg Gly Leu Pro
            315                 320                 325

TCC ACA GCC TCC GGC CGC CTG CGG GGG ACG CAG GAA GTG GCT CCC CCC         1182
Ser Thr Ala Ser Gly Arg Leu Arg Gly Thr Gln Glu Val Ala Pro Pro
        330                 335                 340

ACA CCC CTC ACC CCT ACC TCA CAC ACG GCT AAC ACC TCC CCC CGG CCC         1230
Thr Pro Leu Thr Pro Thr Ser His Thr Ala Asn Thr Ser Pro Arg Pro
    345                 350                 355

GTC TCC GGC ATG GAG AGA GAG CGG AAA GTG AGT ATG CGG CTG CAC CGC         1278
Val Ser Gly Met Glu Arg Glu Arg Lys Val Ser Met Arg Leu His Arg
360                 365                 370                 375

GGG GCC CCC GTC AAC ATC TCC TCG TCC GAC CTC ACA GGC CGA CAA GAT         1326
Gly Ala Pro Val Asn Ile Ser Ser Ser Asp Leu Thr Gly Arg Gln Asp
                380                 385                 390

ACC TCT CGC ATG TCC ACC TCA CAG ATT CCT GGT CGG GTG GCT TCC AGT         1374
Thr Ser Arg Met Ser Thr Ser Gln Ile Pro Gly Arg Val Ala Ser Ser
            395                 400                 405
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GGT CTT CAG TCT GTC GTG CAC CGA TGAGAACTCT CCTTATTGCT GTGAAGGGCA     1428
Gly Leu Gln Ser Val Val His Arg
        410                 415

GACAATGCAT GGCTGATCTA CTCTGTTACC AATGGCTTTA CTAGTGACAC GTCCCCCGGT     1488

CTAGGATCGA AATGTTAACA CCGGGAGCTC TCCAGGCCAC TCACCCAGCG ACGCTCGTGG     1548

GGGAAACATA CTAAACGGAC AGACTCCAAG AGCTGCCACC GCTGGGGCTG CACTGCGGCC     1608

CCCCACGTGA ACTCGGTTGT AACGGGGCTG GGAAGAAAAG CAGAGAGAGA ATTGCAGAGA     1668

ATCAGACTCC TTTTCCAGGG CCTCAGCTCC CTCCAGTGGT GGCCGCCCTG TACTCCCTGA     1728

CGATTCCACT GTAACTACCA ATCTTCTACT TGGTTAAGAC AGTTTTGTAT CATTTTGCTA     1788

AAAATTATTG GCTTAAATCT GTGTAAAGAA AATCTGTCTT TTTATTGTTT CTTGTCTGTT     1848

TTTGCGGTCT TACAAAAAAA ATGTTGACTA AGGAATTCTG AGACAGGCTG GCTTGGAGTT     1908

AGTGTATGAG GTGGAGTCGG GCAGGGAGAA GGTGCAGGTG GATCTCAAGG GTGTGTGCTG     1968

TGTTTGTTTT GCAGTGTTTT ATTGTCCGCT TTGGAGAGGA GATTTCTCAT CAAAAGTCCG     2028

TGGTGTGTGT GTGTGCCCGT GTGTGGTGGG ACCTCTTCAA CCTGATTTTG GCGTCTCACC     2088

CTCCCTCCTC CCGTAATTGA CATGCCTGCT GTCAGGAACT CTTGAGGCCC TCGGAGAGCA     2148

GTTAGGGACC GCAGGCTGCC GCGGGGCAGG GGTGCAGTGG GTGTTACCAG GCAAAGCACT     2208

GCGCGCTTCT TCCCCAGGAG GTGGGCAGGC AGCTGAGAGC TTGGAAGCAG AGGCTTTGAG     2268

ACCCTAGCAG GACAATTGGG AGTCCCAGGA TTCAAGGTGG AAGATGCGTT TCTGGTCCCT     2328

TGGGAGAGGA CTGTGAACCG AGAGGTGGTT ACTGTAGTGT TTGTTGCCTT GCTGCCTTTG     2388

CACTCAGTCC ATTTTCTCAG CACTCAATGC TCCTGTGCGG ATTGGCACTC CGTCTGTATG     2448

AATGCCTGTG GTTAAAACCA GGAGCGGGGC TGTCCTTGCC ACGTGCCAAG ACTAGCTCAG     2508

AAAAGCCGGC AGGCCAGAAG GACCCACCCT GAGGTGCCAA GGAGCAGGTG ACTCTCCCAA     2568

CCGGACCCAG AACCTTCACG GCCAGAAAGT AGAGTCTGCG CTGTGACCTT CTGTTGGGCG     2628

CGTGTCTGTT GGTCAGAAGT GAAGCAGCGT GCGTGGGGCC GAGTCCCACC AGAAGGCAGG     2688

TGGCCTCCGT GAGCTGGTGC TGCCCCAGGC TCCATGCTGC TGTGCCCTGA GGTTCCCAGG     2748

ATGCCTTCTC GCCTCTCACT CCGCAGCACT TGGGCGGTAG CCAGTGGCCA TGTGCTCCCA     2808

ACCCCAATGC GCAGGGCAGT CTGTGTTCGT GGGCACTTCG GCTGGACCCC ATCACGATGG     2868

ACGATGTTCC CTTTGGACTC TAGGGCTTCG AAGGTGTGCA CCTTGGTTCT CCCTTCTCCT     2928

CCCCAGAGTT CCCCCGGATG CCATAACTGG CTGGCGTCCC AGAACACAGT TGTCAACCCC     2988

CCCACCAGCT GGCTGGCCGT CTGTCTGAGC CCATGGATGC TTTCTCAATC CTAGGCTGGT     3048

TACTGTGTAA GCGTGTTGGA GTACGGCGCC TTGAGCGGGT GGGAGCTGTG TGTTGAAGTA     3108

CAGAGGGAGG TTGGGGTGGG TCAGAGCCGA GTTAAGAGAT TTTCTTTGTT GCTGGACCCC     3168

TTCTTGAAGG TAGACGTCCC CCACCCGGAG AGACGTCGCG CTGTGGCCTG AAGTGGCGCA     3228

AGCTTGCTTT GTAAATATCT GTGGTCCCGA TGTAGTGCCC AGAACGTTTG TGCGAGGCAG     3288

CTCTGCGCCC GGGTTCCAGC CCGAGCCTCG CCGGGTCGCG TCTTCGGAGT GCTTGTGACA     3348
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GTCCTTGCCC AGTATCTAGT CCCCGTCGCC CCGTGCAGGA GACGTAGGTA GGACGTCGTG 3408

TCAGCTGTGC ACTGACGGCC AGTCTCCGAG CTGTGCGTTT GTATCGCCAC TGTATTTGTG 3468

TACTTTAACA ATCGTGTAAA TAATAAATTC GGAATTC 3505

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 415 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Met Glu Leu Arg Val Gly Asn Arg Tyr Arg Leu Gly Arg Lys Ile Gly
1 5 10 15

Ser Gly Ser Phe Gly Asp Ile Tyr Leu Gly Thr Asp Ile Ala Ala Gly
20 25 30

Glu Glu Val Ala Ile Lys Leu Glu Cys Val Lys Thr Lys His Pro Gln
35 40 45

Leu His Ile Glu Ser Lys Ile Tyr Lys Met Met Gln Gly Gly Val Gly
50 55 60

Ile Pro Thr Ile Arg Trp Cys Gly Ala Glu Gly Asp Tyr Asn Val Met
65 70 75 80

Val Met Glu Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Asn Phe Cys
85 90 95

Ser Arg Lys Phe Ser Leu Lys Thr Val Leu Leu Leu Ala Asp Gln Met
100 105 110

Ile Ser Arg Ile Glu Tyr Ile His Ser Lys Asn Phe Ile His Arg Asp
115 120 125

Val Lys Pro Asp Asn Phe Leu Met Gly Leu Gly Lys Lys Gly Asn Leu
130 135 140

Val Tyr Ile Ile Asp Phe Gly Leu Ala Lys Lys Tyr Arg Asp Ala Arg
145 150 155 160

Thr His Gln His Ile Pro Tyr Arg Glu Asn Lys Asn Leu Thr Gly Thr
165 170 175

Ala Arg Tyr Ala Ser Ile Asn Thr His Leu Gly Ile Glu Gln Ser Arg
180 185 190

Arg Asp Asp Leu Glu Ser Leu Gly Tyr Val Leu Met Tyr Phe Asn Leu
195 200 205

Gly Ser Leu Pro Trp Gln Gly Leu Lys Ala Ala Thr Lys Arg Gln Lys
210 215 220

Tyr Glu Arg Ile Ser Glu Lys Lys Met Ser Thr Pro Ile Glu Val Leu
225 230 235 240

Cys Lys Gly Tyr Pro Ser Glu Phe Ala Thr Tyr Leu Asn Phe Cys Arg
245 250 255

Ser Leu Arg Phe Asp Asp Lys Pro Asp Tyr Ser Tyr Leu Arg Gln Leu
260 265 270

Phe Arg Asn Leu Phe His Arg Gln Gly Phe Ser Tyr Asp Tyr Val Phe
275 280 285

Asp Trp Asn Met Leu Lys Phe Gly Ala Ser Arg Ala Ala Asp Asp Ala
290 295 300

Glu Arg Glu Arg Arg Asp Arg Glu Glu Arg Leu Arg His Ser Arg Asn
305 310 315 320

Pro Ala Thr Arg Gly Leu Pro Ser Thr Ala Ser Gly Arg Leu Arg Gly
325 330 335

Thr Gln Glu Val Ala Pro Pro Thr Pro Leu Thr Pro Thr Ser His Thr
340 345 350

Ala Asn Thr Ser Pro Arg Pro Val Ser Gly Met Glu Arg Glu Arg Lys
355 360 365

Val Ser Met Arg Leu His Arg Gly Ala Pro Val Asn Ile Ser Ser Ser
370 375 380

Asp Leu Thr Gly Arg Gln Asp Thr Ser Arg Met Ser Thr Ser Gln Ile
385 390 395 400

Pro Gly Arg Val Ala Ser Ser Gly Leu Gln Ser Val Val His Arg
405 410 415

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CTAGATCTAG CTAGACCATG GTAGTTTTTT CTCCTTGACG 40

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CATGCCATGG CACGACCTAG T 21

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

CTAGATCTAG CTAGACCATG GTAGTTTTTT CTCCTTGACG 40

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

GAATCGGGCC GCCGAGATCT CATATGGAGC TGAGAGTC 38

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CCCGGATCTA GCAGATCTCA T 21

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Ala Ser Ser Ser Gly Ser Lys Ala Glu Phe Ile Val Gly Gly Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Arg Ser Met Thr Val Ser Thr Ser Gln Asp Pro Ser Phe Ser Gly Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

TACATCTAGA ATTATGGCGA GTAGCAGCGG C 31

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

AATGGATCCT TAGAAACCTG TGGGGGT 27

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

AATGGATCCT TAGAAACCTT TCATGTTACT CTTGGT 36

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TACATCTAGA ATTATGGAGC TGAGAGTCGG G 31

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GGATCCTCAT CGGTGCACGA CAGACTG 27

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

TACATCTAGA ATTATGGCAC GACCTAGTGG TCGATCG 37

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGGGATCCTA CTTCAGTAGG GGCTG 25

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Arg Ser Gly His Asn Thr Arg Gly Thr Gly Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Arg Leu Gly His Asn Thr Arg Gly Thr Gly Ser Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Ser Ser Arg Pro Lys Thr Asp Val Leu Val Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Lys Ser Asp Asn Thr Lys Ser Glu Met Lys His Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Gly Thr Asp Ile Ala Ala Gly Glu
1 5

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Glu Arg Arg Asp Arg Glu Glu Arg Leu Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Thr Gly Lys Gln Thr Asp Lys Thr Lys Ser Asn Met Lys Gly Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Asp Leu Leu Gly Pro Ser Leu Glu Asp Leu Phe Gly Tyr
1 5 10

CLAIMS

1. An isolated polynucleotide sequence encoding a polypeptide with an amino acid sequence having at least about 35% homology in the protein kinase domain with the polynucleotide encoding *HRR25* protein kinase.

2. The polynucleotide of claim 1 wherein the encoded polypeptide possesses casein kinase activity.

3. The polynucleotide of claim 1 wherein the encoded polypeptide possesses protein-serine/threonine kinase activity.

4. The polynucleotide of claim 1 wherein the encoded polypeptide possesses protein-tyrosine kinase activity.

5. The polynucleotide of claim 1 wherein the encoded polypeptide possess protein-serine/threonine and protein-tyrosine kinase activity.

6. The polynucleotide of claim 1, wherein the polypeptide is characterized as:

a) promoting normal meiotic recombination; and

b) promoting the repair a DNA strand break which occurs at the cleavage site:

CAACAG
GTTGTC .

7. The polynucleotide of claim 1, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of GPSLED (amino acids 86 to 91 in SEQ ID NO: 2), RDIKPDNFL (amino acids 127 to 135 in SEQ ID NO: 2), HIPYRE (amino acids 164 to 169 in SEQ ID NO: 2), and

SVN (amio acids 181 to 183 in SEQ ID NO: 2) and conservative variations thereof.

8. The polynucleotide of claim 1, selected from the group consisting of RNA, mRNA, genomic DNA and cDNA.

9. An antisense polynucleotide according to claim 1.

10. The polynucleotide of claim 1, selected from the group consisting of the DNA sequences of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 23, 30, 31, and 34.

11. An isolated and purified polypeptide encoded by a DNA sequence of claim 10.

12. The polynucleotide of claim 1 wherein the polynucleotide is isolated from organisms selected from the group consisting of *Saccharomyces, Schizosaccharomyces,* human, bovine, porcine, murine, avian and *Drosophila* species.

13. An autonomously replicating DNA vector comprising a DNA according to claim 8.

14. A procaryotic or eukaryotic host cell stably transformed or transfected with a DNA according to claim 8.

15. A method for the production of a polypeptide possessing protein kinase and/or recombination/repair promoting activity comprising growing a host cell according to claim 14 in a suitable nutrient medium and isolating the desired polypeptide from said host cell or from the medium of its growth.

16. A polypeptide product of the method of claim 15.

17. An antibody substance specific for a polypeptide of claim 15.

18. A monoclonal antibody according to claim 17.

19. A method for identifying a composition which modulates the protein kinase and/or recombination/repair promoting activity of an HRR25-like protein comprising:

(a) incubating a system of components comprising the composition and the protein in the presence of a substrate for said protein wherein incubation is carried out under conditions sufficient to allow the components to interact; and

(b) measuring the change in activity of said protein on said substrate.

20. The method of claim 19 wherein the activity is promotion of repair of a DNA double strand break.

21. The method of claim 19 wherein the activity is protein kinase activity.

22. A method of treating a cell proliferative disorder associated with an HRR25-like protein comprising administering, to a subject with the disorder, a therapeutically effective amount of a composition which modulates the activity of the protein.

A.

B.

FIGURE 1

```
Hrr25       --------MDLRVGRKFRIGRKIGSGSFGDIYHGTNLISG----------------EEVA
Ykl/Cki2    ----SSRDDSTIIGLHYKIGKKIGEGSFGVLFEGTNMING----------------VPVA
Yck2/Cki1   -SGSQSRDDSTIIGLHYKIGKKIGEGSFGVLFEGTNMING----------------LPVA
Nuf1        ---MSQRSSQHIVGIHYAVGPKIGEGSFGVIFEGENILHSCQAQTGSKRDSSIIMANEPVA
Hhp1        -------MALDLRIGNKYRIGRKIGSGSFGDIYLGTNVVSG----------------EEVA
Hhp2        -----MTVVDIKIGNKYRIGRKIGSGSFGQIYLGLNTVNG----------------EQVA
CKIα1Hu     MASSSGSKAEFIVGGKYKLVRKIGSGSFGDIYLAINITNG----------------EEVA
CKIα2Hu     MASSSGSKAEFIVGGKYKLVRKIGSGSFGDIYLAINITNG----------------EEVA
CKIα3Hu     MASSSGSKAEFIVGGKYKLVRKIGSGSFGDIYLAINITNG----------------EEVA
Common      ------------G-KYKIGRKIGSGSFGDIY-GTN--NG----------------E-VA

Hrr25       IKLESIRSRHPQLDYESRVYRYLSGGVGIPFIRWFGREGEYNAMVIDLLGPSLEDLFNYCH
Ykl/Cki2    IKFEPRKTEAPQLRDEYKTYKILNGTPNIPYAYYFGQEGLHNILVIDLLGPSLEDLFDWCG
Yck2/Cki1   IKFEPRKTEAPQLKDEYRTYKILAGTPGIPQEYYFGQEGLHNILVIDLLGPSLEDLFDWCG
Nuf1        IKFEPRHSDAPQLRDEFRAYRILNGCVGIPHAYYFGQEGMHNILIIDLLGPSLEDLFEWCN
Hhp1        IKLESTRAKHPQLEYEYRVYRILSGGVGIPFVRWFGVECDYNAMVMDLLGPSLEDLFNFCG
Hhp2        VKLEPLKARHHQLEYEFRVYNILKGNIGIPTIRWFGVTNSYNAMVMDLLGPSLEDLFCYCG
CKIα1Hu     VKLESQKARHPQLLYESKLYKILQGGVGIPHIRWYGQEKDYNVLVMDLLGPSLEDLFNFCS
CKIα2Hu     VKLESQKARHPQLLYESKLYKILQGGVGIPHIRWYGQEKDYNVLVMDLLGPSLEDLFNFCS
CKIα3Hu     VKLESQKARHPQLLYESKLYKILQGGVGIPHIRWYGQEKDYNVLVMDLLGPSLEDLFNFCS
Common      IKLEP-KA-HPQL-YE-RVYKIL-G-VGIP--RWFG--G-YNALVIDLLGPSLEDLF--CG

Hrr25       RRFSFKTVIMLALQMFCRIQYIHGRSFIHRDIKPDNFLMG--VGRRGST------------
Ykl/Cki2    RKFSVKTVVQVAVQMITLIEDLHAHDLIYRDIKPDNFLIGRPGQPDANN------------
Yck2/Cki1   RRFSVKTVLLLADQLITLIEDLHAHDLIYRDIKPDNFLIGRPGQPDANK------------
Nuf1        RKFSVKTTCMVAKQMIDRVRAIHDHDLIYRDIKPDNFLISQYQRISPEGKVIKSCASSSNN
Hhp1        RKFSLKTVLLLADQLISRIEFIHSKSFLHRDIKPDNFLMG--IGKRGNQ------------
Hhp2        RKFTLKTVLLLADQLISRIEYVHSKSFLHRDIKPDNFLM----KKHSNV------------
CKIα1Hu     RRFTMKTVLMLADQMISRIEYVHTKNFIHRDIKPDNFLMG--IGRHCNK------------
CKIα2Hu     RRFTMKTVLMLADQMISRIEYVHTKNFIHRDIKPDNFLMG--IGRHCNK------------
CKIα3Hu     RRFTMKTVLMLADQMISRIEYVHTKNFIHRDIKPDNFLMG--IGRHCNKCLESPVGKRKRS
Common      RRFS-KTVLMLADQMISRIEYIH--DFIHRDIKPDNFLMG---G---N------------

Hrr25       ----------------VHVIDFGLSKKYRDFNTHRHIPYRENKSLTGTARYASVNTHLGIE
Ykl/Cki2    ----------------IHLIDFGMAKQYRDPKTKQHIPYREKKSLSGTARYMSINTHIGRE
Yck2/Cki1   ----------------VHLIDFGMAKQYRDPKTKQHIPYREKKSLSGTARYMSINTHIGRE
Nuf1        NDPNL-----------IYMVDFGMAKQYRDPRTKQHIPYRERKSLSGTARYMSINTHFGRE
Hhp1        ----------------VNIIDFGLAKKYRDHKTHLHIPYRENKNLTGTARYASINTHLGIE
Hhp2        ----------------VTMIDFGLAKKYRDFKTHVHIPYRDNKNLTGTARYASINTHIGIE
CKIα1Hu     ----------------LFLIDFGLAKKYRDNRTRQHIPYREDKNLTGTARYASINAHLGIE
CKIα2Hu     ----------------LFLIDFGLAKKYRDNRTRQHIPYREDKNLTGTARYASINAHLGIE
CKIα3Hu     MTVSTSQDPSFSGLNQLFLIDFGLAKKYRDNRTRQHIPYREDKNLTGTARYASINAHLGIE
Common      ----------------VHLIDFGLAKKYRDPKTHQHIPYRENKSLTGTARYASINTHLGIE
```

FIGURE 2A

```
Hrr25       QSRRDDLESLGYVLIYFCKGSLPWQGLKATTKKQKYDRIMEKKLNVSVETLCSGLPL--EF
Yck1/Cki2   QSRRDDMEALGHVFFYFLRGHLPWQGLQAPNNKQKYEKIGEKKRLTNLYDLAQGLPV--QF
Yck2/Cki1   QSRRDDMEAMGHVFFYFLRGQLPWQGLQAPNNKQKYEKIGEKKRLTNLYDLAQGLPI--QF
Nuf1        QSRRDDLESLGHVFFYFLRGSLPWQGLKAPNNKLKYEKIGMTKQKLNPDDLLLNNAIPYQF
Hhp1        QSRRDDLESLGYVLVYFCRGSLPWQGLAATTKKQKYEKIMEKKISTPTEVLCRGFPQ--EF
Hhp2        QSRRDDLESLGYVLLYFCRGSLPWQGLQADTKEQKYQRIRDTKIGTPLEVLCKGLPE--EF
CKIα1Hu     QSRRDDMESLGYVLMYFNRTSLPWQGLKAATKKQKYEKISEKKMSTPVEVLCKGFPA--EF
CKIα2Hu     QSRRDDMESLGYVLMYFNRTSLPWQGLKAATKKQKYEKISEKKMSTPVEVLCKGFPA--EF
CKIα3Hu     QSRRDDMESLGYVLMYFNRTSLPWQGLKAATKKQKYEKISEKKMSTPVEVLCKGFPA--EF
Common      QSRRDDMESLGYVL-YF-RGSLPWQGLKAPTKKQKYEKIGEKK--T-LEVLC-GLP---EF

Hrr25       -QEYMAYCKNLKFDEKPDYLFLARLFKDLSIKLEYHNDHLFDWTMLRYTKAMVE
Yck1/Cki2   GRYLEIVERSLSFEECPDYEGYRKLLLSVLDDLGETADGQYDWMKLNDGRG
Yck2/Cki1   GRYLEIVERNLSFEETPDYEGYRMLLLSVLDDLGETADGQYDWMKLNGGRG
Nuf1        -ATYLKYARSLKFDEDPDYDYLISLMDDALRLNDLKDDGHYDWMDLNGGKG
Hhp1        -SIYLNYTRSLRFDDKPDAYFRKRLRKDFCRQSEEFNYMLFDWTLKRKT
Hhp2        -T-YMCYTRQLSFTEKPNYAYLMKAFRDLLIRKGYQYDYVFDWMILK
CKIα1Hu     -AMYLNYCRGLRFEEAPDYMYLRQLFRILFRTLNHQYDYTFDWTMLKQKAAQQAASSSGQG
CKIα2Hu     -AMYLNYCRGLRFEEAPDYMYLRQLFRILFRTLNHQYDYTFDWTMLKQKAAQQAASSSGQG
CKIα3Hu     -AMYLNYCRGLRFEEAPDYMYLRQLFRILFRTLNHQYDYTFDWTMLKQKAAQQAASSSGQG
Common      ---YL-Y-R-LSFDEKPDY-YLR-LF--LL--------D--FDWT-L-

CKIα1Hu     QQAQTPTGF
CKIα2Hu     QQAQTPTGFKQTDKTKSNMKGF
CKIα3Hu     QQAQTPTGFKQTDKTKSNMKG
```

FIGURE 2B

A.

B.

FIGURE 1

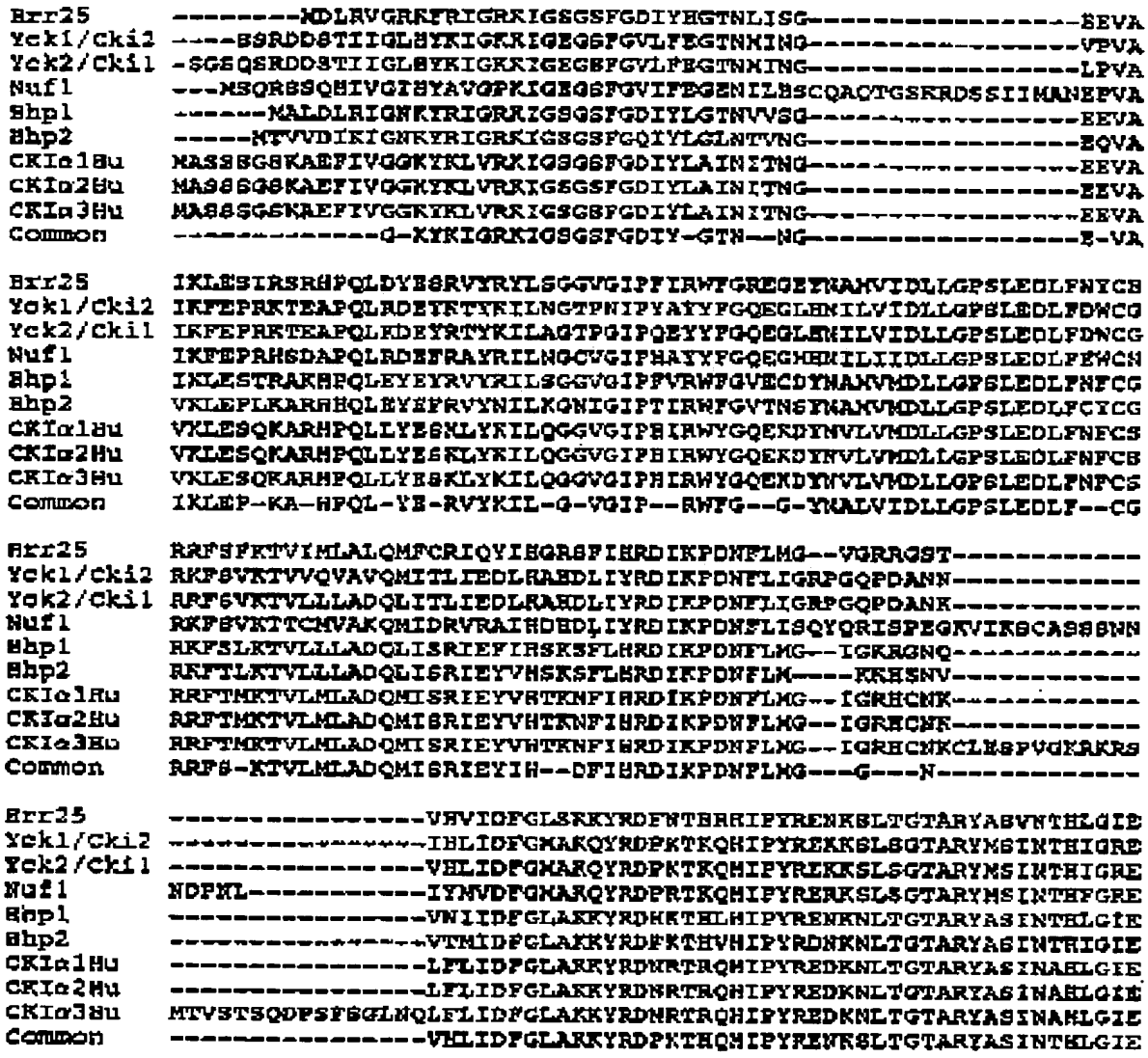

FIGURE 2A

```
Brr25      QSRRDDLESLGYVLIYFCKGSLPWQGLKATTKKQKYDRIMEKKLNVSVETLCSGLPL--EF
Yck1/Ck12  QSRRDDMEALGHVFFYFLRGHLPWQGLQAPNNKQKYEKIGEKKRLTNLYDLAQGLPV--QF
Yck2/Ck11  QSRRDDMEAMGHVFFYFLRGQLPWQGLQAPKNKQKYEKIGEKKRLTNLYDLAQGLPI--QF
Nufl       QSRRDDLESLGEVFTYFLRGSLPWQGLKAPWNKLKYEKIGNTKQKLNPDDLLLNNAIPYQF
Shp1       QSRRDDLESLGYVLVYFCRGSLPWQGLAATTKKQKYEKIMEKKISTPTEVLCRGFPQ--EF
Shp2       QSRRDDLESLGYVLLYFCRGSLPWQGLQADTKEQKYQRIRDTKIGTPLEVLCKGLPE--EF
CKIα1Hu    QSRRDDMESLGYVLMYFNRTSLPWQGLKAATKKQKYEKISEKKMSTPVEVLCKGFPA--EF
CKIα2Hu    QSRRDDMESLGYVLMYFNRTSLPWQGLKAATKKQKYEKISEKKMSTPVEVLCKGFPA--EF
CKIα3Hu    QSRRDDMESLGYVLMYFNRTSLPWQGLKAATKKQKYEKISEKKMSTPVEVLCKGFPA--EF
Common     QSRRDDMESLGYVL-YF-RGSLPWQGLKAPTKKQKYEKIGEKK--T-LEVLC-GLP---EF

Brr25      -QEYMAYCKNLKFDERPDYLFLARLFKDLSIKLEYHNDGLFDWTMLRYTKAMVE
Yck1/Ck12  GRYLEIVERSLSFEECPDYEGYRKLLLSVLDDLGETADGQYDWMKLNDGRG
Yck2/Ck11  GRYLEIVERNLSFEETPDYEGYRMLLLSVLDDLGETADGQYDWMKLNGGRG
Nufl       -ATYLKYARSLKFDEDPDYDYLISLMDDALRLNDLRDDGHYDWMDLNGGKG
Shp1       -SIYLNYTRSLRFDDKPDAYFAKRLRKDFCRQSEEFNYMLPDWTLKRKT
Shp2       -T-YMCYTRQLSFTEKPNYAYLMKAFRDLLIRKGYQYDYVFDWMILK
CKIα1Hu    -AMYLNYCRGLRFEEAPDYMYLRQLFRILFRTLNEQYDYTFDWTMLKQKAAQQAASSSGQG
CKIα2Hu    -AMYLNYCRGLRFEEAPDYMYLRQLFRILFRTLNHQYDYTFDWTMLKQKAAQQAASSSGQG
CKIα3Hu    -AMYLNYCRGLRFEEAPDYMYLRQLFRILFRTLNEQYDYTFDWTMLKQKAAQQAASSSGQG
Common     ---YL-Y-R-LSFDEKPDY-YLR-LF--LL----------D--FDWT-L-

CKIα1Hu    QQAQTPTGF
CKIα2Hu    QQAQTPTGFKQTDKTKSHMKGT
CKIα3Hu    QQAQTPTGFKQTDKTKSHMKG
```

FIGURE 2B

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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C12N 15/54, C07K 15/28, C12N 5/10, 9/12
C12Q 1/48, A61K 37/52, 48/00

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(74) Agent: NOLAND, Greta, E.; Marshall, O'Toole, Gerstein, Murray & Borun, 6300 Sears Tower, 233 South Wacker Drive, Chicago, IL 60606 (US).

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(57) Abstract

Protein kinase mutant and wild-type genes encoding polypeptides of the class heretofore designated "casein kinase I" and useful in screening compositions which may effect DNA double-strand break repair activity are disclosed. Also disclosed are methods using the polynucleotides in cell-proliferative disorders.

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INTERNATIONAL SEARCH REPORT

Internati... Application No
PCT/US 94/00795

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/54 C07K15/28 C12N5/10 C12N9/12 C12Q1/48
A61K37/52 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCIENCE vol. 253 , 30 August 1991 , LANCASTER, PA US pages 1031 - 1034 M. F. HOEKSTRA ET AL. 'HRR25, a putative protein kinase from budding yeast:Association with repair of damaged DNA' see the whole document ---	1,4,6, 11,12
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89 , August 1992 , WASHINGTON US pages 7008 - 7012 ANTHONY DEMAGGIO ET AL. 'The budding yeast HRR25 gene product is a casein kinase I isoform.' see the whole document ---	1,2,6-8, 10-15

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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2

Date of the actual completion of the international search	Date of mailing of the international search report
24 August 1994	05 -09- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Delanghe, L

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA. vol. 89 , October 1992 , WASHINGTON US pages 9454 - 9458 JENNIFER BROCKMAN 'Cell cycle-dependent localization of casein kinase I to mitotic spindles.' see the whole document	1-8, 10-18
P,X	JOURNAL OF BIOLOGICAL CHEMISTRY. vol. 268, no. 9 , March 1993 , BALTIMORE US pages 6394 - 6401 PAUL GRAVES ET AL. 'Molecular cloning ,expression and characterization of a 49-kilodalton casein kinase I isoform from rat testis.' see the whole document	1-8, 10-18
P,X	WO,A,93 01205 (THE SALK INSTIUTE) 21 January 1993 see the whole document	1-21

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US94/00795

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claim 22 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
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3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

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Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

PCT/US 94/00795

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9301205	21-01-93	EP-A- 0594710	04-05-94

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